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Use of Paired Columns in Conjunction with a Quadruple Inlet System in Gas Chromatography

PUBLICATION
199

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PESTICIDES PROGRAM
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THERE is room enough in the oven of many gas chromatographs to accommodate more columns than are supplied with the equipment. It is easy to have multiple injection ports manufactured and increase the number of columns. Thus the productivity of the equipment may be increased by a relatively small investment for additional recorders and the modification of the equipment itself. This merely increases the number of samples that may be run in a given time. However a further modification of the columns permits a real technical improvement whereby aliquots of a single sample may be passed sequentially through different columns to the same detector without the necessity of altering column connections in the oven. The need to use different columns in the identification of unknowns is recognized, but use of unmodified equipment involves delays for change of column and serious difficulty in duplicating temperature and other conditions after the second column has been installed.

This problem has been solved by using an inlet system with four ports and by connecting the outlets of two columns with a tee leading into a single detector and connecting the other two columns by a second tee leading into a second detector. Carrier gas flow to each column was controlled independently by means of gas flow meters. As the column oven was already large enough to accommodate four columns no further modification was necessary.

In this way it was possible to inject a sample first through a polar and then through a non-polar column under identical conditions and thus to obtain more reliable results in less time because the instrument did not have to be shut down for column changes. The other two inlets with a similar column arrangement were operated at the same time using another type of detector. Thus two pairs of columns and two detectors were operated independently of one another, and this increased the versatility of the instrument and doubled its output.

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Determination of Pentachlorophenol in Blood, Urine, Tissue, and Clothing

Small

PUBLICATION

298

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6

Determination of Pentachlorophenol in Blood, Urine, Tissue, and Clothing

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An epidemic with two deaths occurred in a nursery in St. Louis after the use of pentachlorophenol as a mildew preventive with the laundry detergent. Samples of blood, urine, tissue, and clothing from the nursery were extracted with ethyl ether. The ether solution was extracted with 5% sodium hydroxide; the basic solution was acidified and then extracted with benzene. The benzene solution was analyzed by electron capture gas chromatography, using a 3% diethylene glycol succinate column with 2% sirupy phosphoric acid on 60-80 mesh Chromosorb G. Pentachlorophenol was found in the samples in sufficient quantity to account for the epidemic. The presence of pentachlorophenol was confirmed by gas chromatography-mass spectroscopy and by thin layer analysis.

The epidemiology of nine clinically distinct cases of illness in infants in a nursery in St. Louis between April and August 1967 has been described by Armstrong and co-workers (1, 2). This illness was characterized by fever and profuse sweating and rapid recovery after exchange transfusion. Two of the cases were fatal before transfusion was tried.

The causative agent was tentatively identified as a phenol. Because of similarity of the thin layer chromatograms, the phenol was first characterized as hexachlorophene [2,2′-methylene-bis-(3,4,6-trichlorophenol)], a common disinfectant. Later, when a solvent system with better resolving power for this class of phenols was used, the compound was identified as pentachlorophenol, an ingredient of a mildew preventive, Loxene, used with the laundry detergent (3).

This laboratory participated in the investigation to confirm the identity of the agent and to determine quantitatively the amount present in order to see if enough of the agent was present to be responsible for the illness.

Samples of blood and urine from the last child to become ill as well as personal and bed clothing were available. Bed clothing was not that actually used by the child, but was from the same source. Small amounts of tissue from the first fatal case were also available. In addition, blood and urine from exposed but asymptomatic children and their nurses were available.

In 1967, Bevenue and co-workers (4) showed that the methods available for chemical analysis of pentachlorophenol would only detect milligram to microgram quantities. These methods were totally unsuitable for the analysis of blood. In their procedure the methyl ether of the phenol was prepared with diazomethane; the etherified mixture was then analyzed by electron capture gas chromatography (GLC). This procedure was suitable for their work but, at the time the samples were received for the present study, we did not have the diazomethane precursors immediately available and we also preferred to use the derivative as a means of confirmation.

Dr. Morton Beroza (5) suggested a column for the GLC of phenols without derivatization: 3% diethylene glycol succinate treated with 2% sirupy phosphoric acid on Chromosorb G. The use of ^{63}Ni in the electron capture detector permitted the operation of the detector at a higher temperature, and thereby eliminated the difficulties encountered from phosphoric acid column bleed-off when using a tritium detector. When pentachlorophenol in hexane solution was injected on the above column, it was detectable at the 0.02 ng level; a full scale deflection was obtained with 0.5 ng at a 30% recorder pen response with an electrometer input-output setting of 1 × 8 units on an arbitrary potentiometer scale and a nitrogen flow rate of 45 ml/min. Essentially no pentachlorophenol could be detected without use of the phosphoric acid. When 1% phosphoric acid was used a tailing peak was observed. When the acid level was increased to 2%, symmetrical peaks of the desired sensitivity were obtained. Dr. Beroza indicated that the phosphoric acid worked by its common ion effect; it reduced the polarity of the pentachlorophenol, thereby permitting the phenol to pass through the column in about 2 min.

Experimental

Extraction

The urine sample was acidified (pH 5) with 1*N* HCl and then extracted with ether. (The amount of urine sample available varied, so water was added when needed to make a minimum 20 ml solution. About 20 ml urine would require about 5 ml 1*N* HCl for acidification; 20 ml ethyl ether would be used for two extractions, yielding about 40 ml ether extract.) The ether solution was extracted with 1 ml 5% NaOH solution, separated, and washed once with 3 ml distilled water. The water washing was added to the NaOH solution; the NaOH solution was acidified (pH 2) with 5*N* HCl and then was extracted three times with benzene (about 3 ml each). The separated benzene extracts were combined and dried over 1-5 g Na_2SO_4 in a tube; the dried benzene extract was decanted into a graduated centrifuge tube, the Na_2SO_4 layer was washed once with benzene, and the wash was added to the centrifuge tube. Centrifugation was used to break any emulsions which formed. This solution was then ready for injection into the gas chromatograph.

One ml aliquots of serum and blood samples were similarly acidified (pH 5) with 0.1*N* HCl and extracted twice with 3 ml ethyl ether and then treated as described for urine.

Clothing, diapers, and linen were subsampled by taking portions, totaling 3 to 9 sq. in., at random from the cloth. These samples were weighed, placed in 3-6 ml dilute (0.1*N*) HCl, and then extracted twice with 20-50 ml ethyl ether. The ether solutions were processed in the same manner as the urine.

Since most of the sample extracts contained unknown amounts of pentachlorophenol, they were injected in the gas chromatograph immediately after extraction. They were then diluted according to the response received from the first injection. The final extracts were generally 5-6 ml and in many cases had to be diluted greatly (up to 60 fold) to provide an on-scale response on the chromatogram.

A Varian Model 1525 gas chromatograph equipped with a tritium detector and a 5′ × ⅛″ column containing 3% diethylene glycol succinate (DEGS) was used in the initial stages of this work. Later, a Micro-Tek Model 220 gas chromatograph with a ^{63}Ni detector and a 6′ × ¼″ column was used. As mentioned earlier, the ^{63}Ni detector at the higher temperature overcame problems of column bleed of the phosphoric acid. The operating parameters are shown in Table 1.

Using the 4′ Pyrex column with 3% DEGS + 2% sirupy phosphoric acid on 60-80 mesh Chromosorb G, a single, well-defined peak with only slight tailing was obtained with pure pentachlorophenol. However, technical pentachlorophenol had three peaks. The major one was pentachlorophenol itself; the other peaks are unknown but probably are partially chlorinated phenols.

Peak heights from samples were compared with peaks from standard solutions to quantitate the amount of pentachlorophenol in the samples. When spiked samples (not treated with Loxene) were analyzed by the above extraction and determinative procedures, 90 to 100% of added pentachlorophenol (0.0025-0.070 mg/ml) was recovered.

Confirmation of Identity

Three techniques were used to confirm the presence of pentachlorophenol.

The Bowman-Beroza *p*-values (6) were of little use at first because of an unexplained lack of linearity in the ⅛″ columns. However, with the 4′ × ¼″ columns operating at lower temperature, we attained good linearity. Under these conditions, the *p*-value for the hexane-acetonitrile system was 0.222.

The silyl derivative of pentachlorophenol, obtained by the method of Kagan and Mabry (7), gave a well-defined peak at 0.11 and 0.21 min with *p,p′*-DDE at 1 min as a standard. Silanized technical pentachlorophenol showed two peaks that were identical in retention time to two found in the St. Louis samples.

The mass spectral analysis of pentachlorophenol at 20 ev on an LKB Model 9000 gas chromatograph-mass spectrometer gave a molecular ion at m/e 264. This was also the base peak. Only three other major peaks were observed: m/e 229, 200, and 165.

Hexane extracts of diaper, shirt, pad, serum, and urine samples were injected into the mass spectrometer from a 1.0 m × 2.5 mm glass column packed with 3% DEGS + 2% phosphoric

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Table 1. Operating conditions for gas chromatography of samples for pentachlorophenol

Condition	4′ × ¼″ Pyrex Column	5′ × ⅛″ Aluminum Column
Packing support	60–80 mesh Chromosorb G	60–80 mesh Chromosorb G
Liquid phase + added acid	3% DEGS + 2% sirupy phosphoric	3% DEGS + 2% sirupy phosphoric
Oven temp., °C.	150	185
Detector temp., °C.	280	202
Inlet temp., °C.	195	220
Detector	^{63}Ni	^{3}H
Sensitivity	0.02 ng pentachlorophenol	0.1 ng pentachlorophenol

acid. All sample spectra gave the four identical major ions when compared with the pentachlorophenol standard spectrum.

To establish that pentachlorophenol was in fact present in Loxene, 100 mg Loxene was added to 100 ml distilled water, acidified to pH 2.5 with 0.1*N* HCl, and then extracted twice with 100 ml hexane. The hexane extract was evaporated down to ca 10 ml, dried over a little Na_2SO_4, and transferred to a conical centrifuge tube. Then IR pellet-grade KCl was added and the remaining ether was carefully evaporated. The residue-salt was pressed into a pellet for infrared spectrophotometric analysis. The spectrum was almost identical to that obtained from the pentachlorophenol standard, except for some extraneous absorption due to impurities not removed during the partitioning steps. The similarity confirmed the presence of pentachlorophenol in Loxene.

Animal Studies

Blood

To determine whether the level of pentachlorophenol in serum was consistent with illness, five female, white Sherman strain rats were dosed with 100 mg/kg pentachlorophenol in peanut oil solution by stomach tube. Three of the five rats had a 24 hr survival, and a composite sample of their blood was made from blood obtained by heart puncture; the sample contained 37.7 ppm pentachlorophenol. The blood of one rat not knowingly exposed to pentachlorophenol was used as a control; it had an apparent pentachlorophenol level of 0.39 ppm.

Urine

To study the level of Loxene and pentachlorophenol in urine to be expected from rats given sublethal doses, one group of five rats was dosed with 100 mg technical pentachlorophenol/kg. Another group of five rats was given 50 mg Loxene/kg. Even though Loxene was administered at a lower level than pentachlorophenol because of its toxicity, this dosage caused diarrhea, contaminating urine with feces so that the urine could not be analyzed. Some of the pentachlorophenol could have passed through with the feces without being absorbed. In the pentachlorophenol-treated group, all five rats had a 24 hour survival; the combined urine sample contained 35.0 ppm pentachlorophenol. The urine of the control rat did not contain pentachlorophenol.

Results and Discussion

The last child to become ill, C. A., was given an exhange transfusion on August 30, 1967. Blood and urine from this child were the only samples of this type available to us because samples from previous cases had been discarded. Table 2 presents pentachlorophenol levels in urine and serum from this patient.

Pathological examination of tissue samples from the first child to die (T. H.) yielded no clues to the illness, but the small amounts

Table 2. Analysis of serum and urine of one child, C. A. (born Aug. 21, 1967; became ill Aug. 29, 1967)

Sample	Date	Pentachlorophenol, ppm
Serum	Aug. 30 (taken during exchange)	118.0
Serum	Aug. 30 (post-exchange)	64.6
Serum	Aug. 31 (day after exchange)	31.2
Urine	Sept. 2	2.44

Table 3. Analysis of tissue of infant, T. H. (born May 9, 1967; died May 15, 1967)

Sample	Wt, g	Pentachlorophenol, ppm
Kidney	0.303	27.6
Adrenal	0.031	27.3
Heart and blood vessel	0.633	21.3
Fat	0.747	33.8
Connective tissue	0.160	26.7

Table 4. Analysis of serum and urine from six exposed but asymptomatic infants

Infant	Date, Sept. 1967	Pentachlorophenol, ppm	
		Serum	Urine
D. M.	2	22.4	
	6	26.1	
J. F.	6	25.6	
	8		0.564 (12 pm–7 am)
	8		0.276 (7 am–1 pm)
K. M.	5	15.94	
	6	10.8	0.962 (12 noon–12 midnight)
	7	7.1	0.535 (12 midnight–12 noon)
C. J. B.	6	15.90	
	7	12.6	0.232 (7:45 pm)
	8		0.084 (12 midnight–12 noon)
			0.104 (4 pm)
C. H.	8	21.05	
	9		0.798 (1 am–9 am)
	9		0.155 (12:30 pm–9 pm)
	9–10		0.487 (9:30 pm–9 am)
	10		0.033 (9 am–11 am)
S. I.	9	15.6	
	10		0.004 (9 am–9 pm)
	11		0.022 (9 am–9 pm)
	11		0.017 (9 pm–9 am)
	12		0.023 (5 am–11:30 am)

available were analyzed for phenols; *see* Table 3.

When the infant C. A. became ill on September 29, there were a number of other asymptomatic children in the nursery. Table 4 presents serum and urine levels of pentachlorophenol from six of these infants.

After pentachlorophenol had been detected and its identity established beyond doubt in tissue, blood, and urine from infants in the nursery, it was necessary to determine the manner of exposure. Linen used by the ill child, C. A., and clean linen from the storage closet were analyzed; *see* Table 5.

Use of Loxene, the suspect source of pentachlorophenol, was discontinued after the illness of C. A., and new linens were purchased. About 2 weeks later, samples from children born since the last incident were made available for analysis. Results are presented in Table 6.

Since the nurses handled the linen to which the infants were exposed, their sera were analyzed for pentachlorophenol in order to determine if such exposure had produced detectable levels of the phenol in their blood. As can be seen in Table 7, values varied from 12.6 to 1.73 ppm.

Serum and urine samples for controls were obtained from two infants in another St. Louis hospital. Surprisingly, these showed small but detectable amounts of pentachlorophenol. Results of these analyses are given in Table 8. Since apparently unexposed children had now yielded detectable amounts of pentachlorophenol in their blood it was necessary to search elsewhere for controls. Cord blood from healthy infants in Atlanta was negative for pentachlorophenol, as shown in Table 9.

Table 5. Analysis of linens and clothing for pentachlorophenol

Sample	Pentachlorophenol, ppm	Sample	Pentachlorophenol, ppm
Used Linen			
Crib pad	48.9	Muslin	28.0
Shirt	73.8	Muslin	11.5
Diaper	172.0	6 diapers	85.3
Diaper	109.6	6 shirts, 1 pillow case, and 1 bed pad	26.6
Pillow case	62.5		
Clean Linen from Storage Closet			
Diaper	26.4	Shirt	79.0
Diaper	44.26	Shirt back	224.0
Diaper	77.40	Shirt front	1950.0
Diaper	99.69	Crib pad	1787.0
		Underpad	140.0

Table 6. Pentachlorophenol levels in serum and urine from infants after discontinuation of use of Loxene

	Pentachlorophenol			
Infant	Serum, ppm	Date, Sept. 1967	Urine, ppm	Date, Sept. 1967
K. D.			0.035	22
J. M.	17.2	21	0.045	22
T. T.	15.4	22	0.088	22
G. M.			0.633	22
H. B.			0.022	22
C. M.	3.89	22	0.023	27
L. P.	3.36	22	0.007	27
P. D.	3.88	24	0.007	27
Carol A.	17.5	25	0.034	27
L. A. D.	3.32	27		

Acknowledgments

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Table 7. Pentachlorophenol levels found in serum of nurses from St. Louis nursery

Nurse	Date, Sept. 1967	Pentachlorophenol, ppm
C. M.	6	3.32
Mrs. K.	6	5.45
M. D.	8	12.6
O. M.	6	1.73
E. M.	7	3.92

Table 8. Pentachlorophenol levels found in serum and urine of control infants; samples were taken on Sept. 6, 1968

Infant	Sample	Pentachlorophenol, ppm
T. B.	Serum	0.459
T. B.	Urine	0.004
T. HL.	Serum	0.069
T. HL.	Urine	0.007

Table 9. Analysis of cord blood from healthy infants[a]

Infant	Sample Size, ml	Pentachlorophenol, ppm[b]
J. L. G.	2.0	<0.016
J. Mc.	1.3	<0.029
A. R.	2.0	<0.016

[a] This blood was obtained in Grady Hospital, Atlanta, Ga. (Nov. 17, 1967) and used as a check on the method of analysis.

[b] All samples were blank; the numbers represent the limit of definition.

REFERENCES

(1) Armstrong, R. W., Eichner, E. R., Klein, D. E., Barthel, W. F., Bennett, J. V., Jonsson, V., Bruce, H., and Loveless, L. E., in preparation.

(2) Armstrong, R. W., in preparation.

(3) Anonymous, *Morbidity Mortality Weekly Rept.* **16**, 334–335 (1967).

(4) Bevenue, A., Wilson, J. R., Potter, E. F., Sang, M. K., Beckman, H., and Mollet, G., *Bull. Environ. Contam. Toxicol.* **1**, 257–266 (1966).

(5) Beroza, M., U.S. Department of Agriculture, Beltsville, Md., personal communication, 1967.

(6) Bowman, M. T., and Beroza, M., *This Journal* **48**, 1943–1952 (1965).

(7) Kagan, J., and Mabry, T. J., *Anal. Chem.* **37**, 288–289 (1965).

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Chamblee, Georgia 30341

Automation of Pesticide Residue Analysis

Die Automatisierung der Analyse von Pestizid-Rückständen

Automatisation de l'analyse des résidus de pesticides

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Summary: The routine analysis of pesticide residue levels in biological tissue by gas-liquid chromatography is greatly facilitated by the use of computers to reduce computation time. Modern systems can be readily adapted to small computers. The nature of gas chromatographic signals permits the insertion of digitizers between the gas chromatograph and the computer. The resulting system permits completely automatic retrieval of data; thus time is saved and operating efficiency of the analytical laboratory is increased.

Zusammenfassung: Die Routineanalyse des Gehalts an Pestizid-Rückständen in biologischem Gewebe durch Gas-Flüssig-Chromatographie wird durch den Einsatz von Computern zur Einsparung von Rechenzeit wesentlich erleichtert. Moderne Systeme können leicht an kleine Computer angepaßt werden. Die Art der gas-chromatographischen Signale gestattet die Einfügung von Digitalisierungs-Gliedern zwischen Gas-Chromatographen und Computer. Mit dem entstandenen System läßt sich die Datensuche vollständig automatisieren; auf diese Weise wird Zeit gespart, und die Leistungsfähigkeit des analytischen Labors wird erhöht.

Sommaire: La determination de routine des teneurs en résidus de pesticides dans des tissus biologiques par chromatographie gaz-liquide est notablement facilitée par l'emploi d'ordinateurs; ceci réduit la durée du calcul. Des systèmes modernes sont faciles à adapter à de petits ordinateurs. La nature des signaux émis en chromatographie en phase gazeuse permet l'insertion de membres de digitalisation entre le chromatographe en phase gazeuse et l'ordinateur. Le système ainsi obtenu permet la recherche tout à fait automatique des données; ainsi on gagne du temps, et l'efficacité du laboratoire analytique est augmentée.

Introduction

Methods for handling gas chromatographic data have not progressed as rapidly as the application of gas chromatography as an analytical tool. The result of the lack of progress in data handling is a growing volume of recorded data being held for reduction to meaningful information. The percentage of time spent by the analyst doing calculations has continued to increase. The analyst identifies the peak on the chromatogram, determines the area of the identified peak, and reduces the area of the sample peak to some statement of quantity relative to a set of responses for standards.

Recent progress in the application of computers to determination of low levels of pesticide residues in biological tissue is a promising aid to the analyst. Systems are now available that permit conversion of the analog signals to digital signals. The converted analog signal can then be handled by computer for final printout of resulting data without delay.

System

The detection of a compound by a gas chromatograph results in the production of an analog voltage signal. An available analog signal can be modified in several ways to render it suitable for computer interfacing. Online instrument-computer modifications require that the analog signal is digitized prior to interfacing with the computer (Fig. 1), requiring digital integrators for each signal source [1]. The digitized signal is then interfaced to the computer, allowing the computer to handle the data channel by set priority. This method involves not only the cost of interfacing but requires the use of more bulk memory for channel reference data storage and channel selection routines. An alternative method is the magnetic tape analog-to-digital system (2) in which a tape playback system supplies data to the digital integrator interfaced with the computer (Fig. 2). The recording device receives the analog signal, converts it to a digital signal, and impresses it on magnetic tape. The magnetic tape is replayed on the playback system to yield the desired results through the integrator-computer system (Fig. 2).

The system used in this laboratory is the magnetic tape playback system. It employs the CRS 40/50 Data Processor *), which is composed of the CRS 42RSID tape recorder, CRS 40 TSR tape playback deck, CRS 11 HSB/42 digital integrator, and CRS 50 digital data processor,

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*) Use of trade names is for identification purposes only, and does not constitute endorsement by the Food and Drug Administration or the Department of Health, Education, and Welfare.

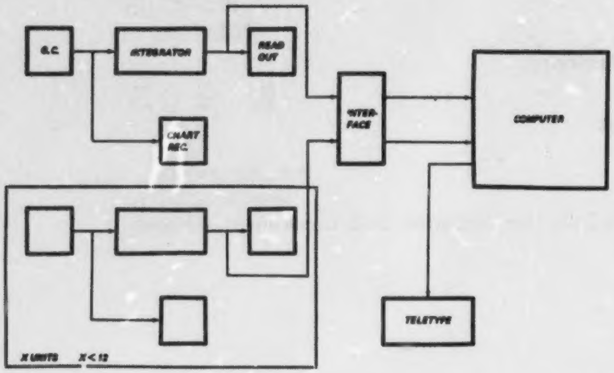

Fig. 1

- Block diagram of online computer system which shows parallel interfacing of up to 12 integrators to the computer.

- Blockdiagramm eines on-line Computer-Systems mit Parallel-Interface für bis zu 12 Integratoren zum Computer.

- Schéma d'un système d'ordinateur utilisé «on-line», avec connexion à l'ordinateur d'un nombre d'intégrateurs pouvant aller jusqu à douze.

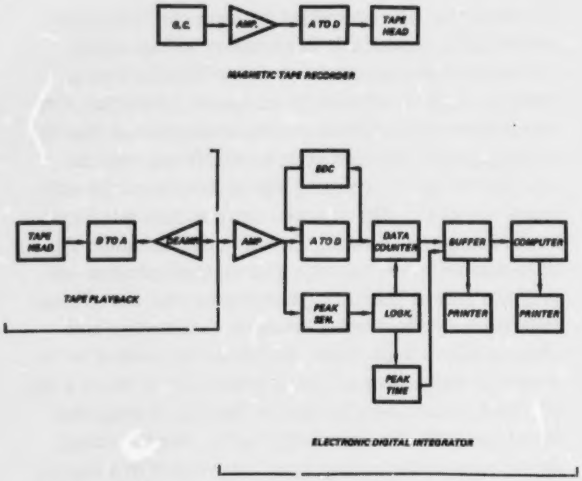

Fig. 2

- Block diagram of magnetic tape playback system which shows a single integrator interfaced to the computer and the playback system for signal conversion and signal introduction to the integrator.

- Blockdiagramm eines Magnetband-Playback-Systems mit einem einzigen durch Interface an den Computer angeschlossenen Integrator und dem Playback-System zur Signalumwandlung und Signalgebung an den Integrator.

- Schéma d'un système «playback» à bande magnétique, avec un seul intégrateur branché à l'ordinateur et le système «playback» pour la conversion des signaux et introduction des signaux dans l'intégrateur.

which are produced by Infotronics Corporation, Houston, Texas. Direct communication with the computer is achieved through an ASR 33 teletypewriter. It no longer is necessary to quantitate the analog signal impressed on a strip chart with this system. Successful digitization is achieved and the computer handles the signal readily.

Data Handling

The analog signal is generated in the GC electrometer when a component passes through the detector. The signal is input to a magnetic tape recorder which converts it to a frequency signal proportionate to the analog signal amplitude and records the frequency signal on magnetic tape. The tape is then played back to the integrator which is interfaced directly to the computer. The integrator generates a time signal for component retention time (Table I), a count rate for peak size, (Table I), and a stop-go signal. The computer starts with a go signal, receives the integrator signal, compares it with a data table (Table II), which has been entered by means of paper punch tape, and computes and tabulates the data output (Table III) through the ASR 33 teletypewriter. Table III is a reproduction of the computer printout of an analysis of a fat sample. The fat sample is one of a large number of samples analyzed in a study being prepared for publication. The type 5 **) mathematical computation, the result of the joint effort of chromatographers in this laboratory and computer programmers at Infotronics Corporation, handles the calculations and renders the printout in parts per million concentration.

Evaluation

The change in rate of data output more than justifies the small cost of the small (4K) computer. Not only is the laboratory efficiency increased by computerization, but the reproducibility of data handling is increased through the usage of electronic sensing devices rather than human sensory devices. The improved sensing of electronics is particularly evident in the slope, peak, and trip level sensing circuits operating in conjunction to detect the departure of a signal from the baseline; with this system, the interpreter is freed from decisions in extrapolating the slopes of the peak traces.

The recorder-playback-integrator-computer system operating in our laboratory is at present being successfully used for data reduction of pesticide analyses. The gas chromatographer can devote more of his time to handling other problems associated with his work.

The successful reduction of data depends on how well the gas chromatograph maintains a fixed state of operation. Constant response factors (mass/count) must be maintained because if changes occur, the computer has no accurate conversion factors for sample response. The parameters affecting retention time and peak resolution must also be maintained constant; any changes can seriously impair

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**) One of several computations handled by the Mark IIC Executive Program, John Heinz, Infotronics Corporation, Houston, Texas.

the effectiveness of the computerized system. The computer labels peaks by comparisons of the retention times of predetermined reference peaks and by the relative retention times of the remainder of the eluting peaks in a given determination. Peak labeling accuracy also depends on the quality of peak resolution. If the distribution curves of sample components overlap in time, the reference table used by the computer must be changed to reflect the most recent state of operation. Without this "state of function" maintenance, the computer printout is useless.

Table I. Integrator output format in ASR 33 teletype language

Time	Area
50	35534
78	8487
100	6700
191	1311
231	1538
321	21379
406	22653
506	33662
528	13336
789	22496
954	20703
1039	16538
1328	17470
1484	16881
1823	18892
1990	14184

Table II. Computer reference table format of the Mark IIC program *)

```
6236
GC ANAL
11
1
7100
5
1039, 50, 8
@
ABHC,  2.447, 321
GBHC,  2.360, 406
HHDN,  4.174, 481
BBHC,  2.451, 506
DBHC,  3.757, 528
IDDE,  2.360, 789
HE,    2.887, 954
2DDE,  3.113, 1039
1DDT,  3.180, 1328
HEOD,  2.906, 1483
2DDD,  2.595, 1821
2DDT,  3.727, 1990
@
8, 1.0
@
```

*) Original reference table format allowed only x.xxx entry (2.447 above), which rendered concentration printout in data table to be x10^{-4}. Changes in the "cleaned-up" program allowed x.xxxxxxxxx entry (0.0002447) which rendered concentration printout in data table to be x 1.

Table III. Computer output *)

```
DATE
TIME

SAMPLE NO.   0000
SAMPLE TYPE   11        GC ANAL

ANALYZER 01
ANALYSIS TYPE    5
INPUT CHANNEL  11
DILUTION FACTOR          9.500
REL. TIME CORR.           .994

AREA     TIME     CONC.        CONSTITUENT

  532     248     5054.000     UNKNOWN
  842     271     3188.001     ABHC
    7     360       66.500     UNKNOWN
   73     418     1636.562     GBHC
 8784     479     2320.024     HHDN
67867    1040     7872.122     2DDE
10440    1357     4032.019     1DDT
24854    1488    14112.041     HEOD
 4063    1847     1816.006     2DDD
32771    2002    12992.070     2DDT
```

*) Computer analysis print-out format with concentration values relative to x.xxx values from Table I.

Esamples: ABHC, 2.447, 321 yields CONC = 3188.001
ABHC, 0.0002447, 321 yields CONC = 0.3188

Extension

The computer system has been found to work well enough to consider extension of the system to accommodate data retrieval of mass spectrometric data and infrared spectrophotometric data. The computer, as it is now operated, would require a data break facility, a 32 K disk memory, and a high speed information read-in modification. In addition to the time saving in the GC laboratory the instrument would greatly reduce the working time of infrared spectrophotometrists and mass spectrometrists.

References

[1] *Barrett, A. T.*, III: The Integrator/Computer System for Gas Chromatography Data Automation, Infotronics Corporation, Houston, Texas, 1968.

[2] *Sadtler, P., Schwartz, W. F.:* Laboratory Management, 6 (6), 22 (1968).

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Adsorption Chromatography of Alkaloids of Fumaria Officinalis

Adsorptions-Chromatographie der Alkaloide von Fumaria Officinalis

Chromatographie d'adsorption des alcaloïdes de Fumaria officinalis

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Summary: Seven alkaloids of Fumaria officinalis, difficult to separate by partition chromatography and countercurrent distribution, were separated by column chromatography using silica gel developed with mixtures of propanol or methanol with nonpolar solvents. Suitable solvent systems for column chromatography were chosen on the basis of thin layer chromatography data.

Zusammenfassung: Sieben Alkaloide aus Fumaria officinalis, die durch Verteilungschromatographie und durch Gegenstromverteilung schwierig zu trennen sind, ließen sich durch Säulenchromatographie an Silicagel mit Mischungen von Propanol oder Methanol mit unpolaren Lösungsmitteln trennen. Passende Lösungsmittelsysteme wurden aufgrund von Erfahrungen mit der Dünnschicht-Chromatographie ausgewählt.

Sommaire: Sept alcaloides de Fumaria officinalis, qui sont difficiles à séparer par chromatographie de partage et par distribution à contre-courant, ont été séparés par chromatographie sur colonne, en utilisant du gel de silice et en développant par des mélanges de propanol ou méthanol avec des solvants non-polaires. Des systèmes solvants appropirés à la chromatographie sur colonne ont été choisis sur la base de données obtenues en chromatographie sur couche mince.

In an earlier investigation carried out in this laboratory, *Wawrzynowicz* and coworkers [1] analysed the alkaloid extracts of Fumaria officinalis by the "moist buffered paper" technique and established the presence of seven alkaloids, six of which were identified by chemical tests and comparison with standards such as stylopine, sinactine, aurotensine, F_{37}, protopine and cryptocavine. The seventh alkaloid behaved as a phenolic compound and therefore was tentatively identified as F_{38} [2, 3]; however, the conclusion requires still further confirmation.

The results obtained with the "moist buffered paper" technique, in view of the partition mechanism and analogy with Craig's countercurrent distribution [4], were utilized for the determination of optimal conditions of preparative separation by the latter method. It was found [5] that the alkaloids are readily separable by countercurrent distribution into two groups of alkaloids, those of protopine and protoberberine. As isolation of individual alkaloids within these groups by countercurrent distribution would require large number of transfers in view of the close similarity of molecular structure of alkaloids belonging to either group, such an isolation remained an open question. In view of the differing mechanism of adsorption chromatography the thin layer technique was applied in the present investigation.

Experimental

The herb of Fumaria officinalis was extracted with ethanol. After evaporation of the solvent, the alkaloids were extracted with 1 N HCl. The acidic extract was alkalized with 10 % ammonia and the alkaloids extracted with ethyl ether and then with chloroform. The aqueous solution was further alkalized with 5 % NaOH and extracted to remove any quaternary bases. The organic extracts were purified several times by reextraction with acidic aqueous solutions, alkalization and extraction with organic solvents.

The extract was chromatographed as in the preceding papers [6, 7] using Kieselgel G (Merck, Darmstadt) as adsorbent. The alkaloids were detected with Dragendorff's reagent, bis-diazotized benzidine or concentrated sulphuric acid. The use of the Wachtmeister's reagent and H_2SO_4 was found to be advantageous in view of their selective reaction with some of the alkaloids so that additional criteria of identification were obtained. The experiments were carried out at 21 ± 1 °C. The R_F values for several systems, in which best separations were obtained, are given in Table I.

Column chromatography

Satisfactory separation of the first three alkaloids (stylopine, sinactine and aurotensine) in the system cyclohexane-n-propanol, and the remaining alkaloids (F_{37}, G-probably F_{38}, protopine and cryptocavine) in the system carbon tetrachloride – methanol (Table I) gave ground to expect good separation of the alkaloids by column adsorption chromatography. The solvent systems for column chromatography were chosen after preliminary experiments using thin layers prepared from silica used for the pre-

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Multiresidue Procedure for Halo- and Nitrophenols. Measurement of Exposure to Biodegradable Pesticides Yielding these Compounds as Metabolites

PESTICIDE
PUBLICATION
614

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0.0001 to 0.01

Environmental Protection Agency
Chamblee, Georgia 30341

The urinary level of phenolic compounds may be the key for establishing an index of exposure to pesticides containing this moiety as an easily hydrolyzed or metabolized portion of the molecule. A method has been developed for quantitating ten halo- and nitrophenols in rat urine which could result from exposure to and subsequent metabolism and excretion of a broad spectrum of pesticides. The procedure involves acid hydrolysis, extraction, derivatization, silica gel chromatography, and electron-capture gas chromatography. Male rats fed pesticidal compounds containing halo- and nitrophenol moieties at levels varying from factors of 10^{-5} to 10^{-2} of the LD_{50} were used to establish the usefulness of this procedure for determining the extent of exposure to the biodegradable pesticides.

Determination of the urinary excretion of halo- and nitrophenol metabolites of biodegradable pesticides is of increasing interest to those involved in pesticide epidemiology. Metabolism studies (Menzie, 1969) of the biodegradable pesticides EPN, fenitrothion, dicapthon, methyl bromophos, C-9491, Dursban, DNOC, PCP, VC-13, and ronnel, which contain halo- or nitrophenol substituent groups in their molecular structures, indicate that these phenols are the major urinary metabolites. The purpose of this investigation was to develop a multiresidue method for the determination of low levels of halo- and nitrophenols in urine. The method is based on electron-capture gas chromatography of ethyl ether derivatives of the phenols (Bradway and Shafik, 1971; Shafik *et al.*, 1971a). Such a procedure may be of value in developing an exposure index to biodegradable pesticides based on levels of these urinary phenols.

EXPERIMENTAL SECTION

Apparatus and Equipment. A Micro-Tek 220 gas chromatograph equipped with tritium foil electron-capture detector was used. A glass U-shaped column, 6 ft × ¼ in., was packed with 4% SE-30/6% QF-1 on 80/100 mesh Chromosorb W (HP). The gas chromatographic column was operated under the following parameters: nitrogen carrier gas flow rate, 60 ml/min; column temperature, 175°; inlet, 210°; detector, 210°; transfer line, 240°. Chromatographic columns were size 22, Kontes K-420100. Concentrator tubes were 25 ml, Kontes K-570050. Condensers were Kontes K-286810. Nitrogen evaporator was equipped with a water bath maintained at 40°. N-Evap was obtained from Organomation Associates, Worcester, Mass.

Reagents. The following were used: *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich Chemical Co., Milwaukee, Wis.); ethylating reagent (Stanley, 1966; Shafik *et al.*, 1971a); and silica gel, Woelm, activity grade I (Waters Associates, Inc., Framingham, Mass.). Dry adsorbent for 48 hr at 170° and store in a desiccator. Prepare daily 2 g of deactivated silica gel for each chromatographic column by adding 40 µl of benzene-extracted deionized or distilled water for each 2 g of dried silica gel in a tightly stoppered container. Rotate container until the water is evenly distributed throughout the adsorbent. Allow to equilibrate for 2 to 3 hr with periodic shaking. Prepare the chromatographic columns just before use. Deionized or distilled water used for deactivating the silica gel must be extracted twice with benzene. Prepare each day the anticipated amount of deactivated silica gel to be used. Larger amounts may be prepared by using the same ratio of water to dried silica gel.

Preparation of Standard Solutions. The phenolic compounds were 95+% pure and a mixture of the ten compounds was prepared at the final concentrations as follows: 2,4-dichlorophenol (2,4-DCP), 0.4 µg/ml; 2,4,5-trichlorophenol (2,4,5-TCP), 0.1 µg/ml; and 3,5,6-trichloro-2-pyridinol (3,5,6-TCPyridinol), 0.1 µg/ml, Dow Chemical Co., Midland, Mich.; 3,6-dichloro-4-iodophenol (3,6-DCIP), 0.05 µg/ml, Ciba Agrochemicals Co., Summit, N. J.; 2,5-dichloro-4-bromophenol (2,5-DCBrP), 0.02 µg/ml, Cela, Ingelheim, West Germany; pentachlorophenol (PCP), 0.01 µg/ml, and *p*-nitrophenol (PNP), 0.2 µg/ml, Aldrich Chemical Co., Milwaukee, Wis.; *p*-nitro-*m*-cresol (PNC), 1.2 µg/ml, Chemagro Corp., Kansas City, Mo.; 2-chloro-4-nitrophenol (2-C-4NP), 0.08 µg/ml, American Cyanamid Co., Princeton, N. J.; 2,4-dinitro-6-methylphenol (DNOC), 0.4 µg/ml, Chemical Insecticide Corp., Edison, N. J. These compounds are the corresponding phenolic metabolites of pesticides listed in Column 1, Table II.

Weigh 10 mg of each of the ten analytical standards into separate 10-ml volumetric flasks. Add 5 ml of benzene to each flask and swirl the flask until the compound dissolves. In a well-ventilated hood, wearing disposable gloves, add diazoethane dropwise with a disposable pipet until a definite yellow color persists. Allow the solution to stand 15 min, and then bubble dry nitrogen through the solution until the yellow color disappears (5–10 min). Dilute to volume with benzene. From these ethylated stock standards, prepare a mixture of the ten compounds at the final concentrations listed above.

Chromatographic Behavior of the Ten Ethyl Ethers on a Silica Gel Column. Pipet an aliquot of the mixture of the ten ethylated compounds into a 15-ml graduated centrifuge tube. Evaporate the benzene to a volume of 0.3 ml using the nitrogen evaporator, and add 1.7 ml of hexane. To a chromatographic column lightly plugged with glass wool, add the 2.00 g of the partially deactivated silica gel and top with 1.5 g of anhydrous sulfate. Prewash the column with 10 ml of hexane and discard the hexane eluate. Transfer the standard mixture quantitatively to the column using 8 ml of the 20% benzene-in-hexane solution and collect this fraction in a 15-ml centrifuge tube (fraction I). Elute the column with 10 ml of 40% benzene-in-hexane (fraction II), followed by 10 ml of 60% benzene-in-hexane and 10 ml of 80% benzene-in-hexane, collecting the 20 ml in a 25-ml concentrator tube (fraction III). Elute the column with 10 ml of benzene and collect this fraction in a 15-ml centrifuge tube (fraction IV). Inject 5–10 µl from each fraction into the gas chromatograph to establish the elution pattern of the ten ethyl ethers from the silica gel column.

Under our laboratory conditions, fraction I (20% benzene-in-hexane) contained the ethyl ethers of 2,4-DCP, 2,4,5-TCP, 3,5,6-TCPyridinol, 3,6-DCIP, 2,5-DCBrP, and

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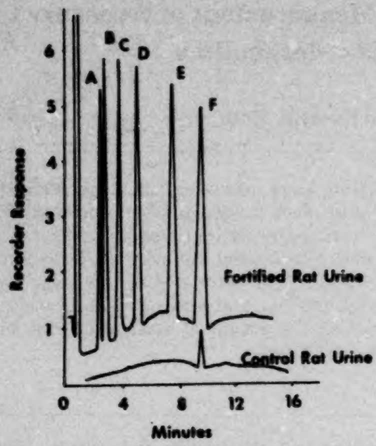

Figure 1. Chromatogram of the 20% benzene-in-hexane fraction of fortified and control rat urine. A. 2,4-DCP, 0.8 ppm; B. 3,5,6-TCPyridinol, 0.05 ppm; C. 2,4,5-TCP, 0.03 ppm; D. 2,5-DCBrP, 0.02 ppm; E. 3,6-DCIP, 0.03 ppm; F. PCP, 0.02 ppm.

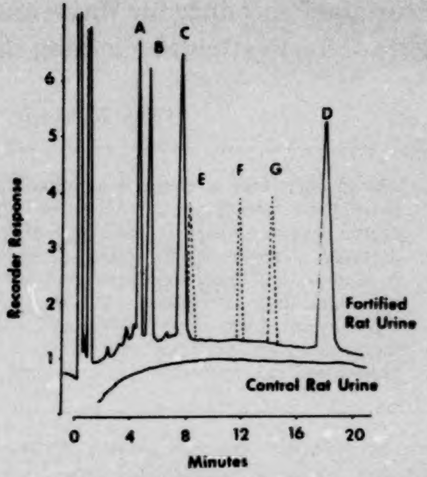

Figure 2. Chromatogram of the 60–80% benzene-in-hexane fraction of fortified and control rat urine: A. PNP, 0.1 ppm; B. PNC, 0.4 ppm; C. 2-C-4NP, 0.04 ppm, D. DNOC, 0.16 ppm; E. 2,4-D; F. 2,4,5-TP; G. 2,4,5-T.

PCP. The ethyl ethers of PNP, PNC, 2-C-4NP, and DNOC eluted in fraction III (60 and 80% benzene-in-hexane). Occasionally, traces of DNOC are found in fraction IV (benzene). Fraction II (40% benzene-in-hexane) did not contain any of the derivatives.

Elution patterns may vary from one laboratory to another, depending on the temperature and relative humidity. It is therefore necessary to establish an elution pattern under local conditions before attempting to analyze samples.

Analysis of Urine. Pipet 1 to 5 ml (the actual volume to be determined by the anticipated residue level) of urine into a 25-ml concentrator tube. Add dropwise a volume of concentrated HCl equal to one-fifth the amount of urine, and mix well. Fit a stoppered reflux condenser to the tube and heat in a boiling water bath for 1 hr while cooling the condenser with circulating ice water. Remove from the bath, cool, and rinse the sides and tip of the condenser with a total of 2 ml 0.1 *N* NaOH. Add 3 ml of anhydrous ethyl ether to the tube and mix contents vigorously on a Vortex mixer for 2 min; then centrifuge and transfer ethyl ether layer to a 15-ml centrifuge tube with a disposable pipet. Repeat the extraction with an additional 3-ml volume of ethyl ether and add the second ethyl ether extract to the centrifuge tube.

Add diazoethane dropwise with a disposable pipet until the yellow color persists. Let the solution stand 15 min; then bubble clean dry nitrogen through the solution to remove excess reagent. Concentrate the ethylated urine extract to approximately 0.3 ml, using the nitrogen evaporator. Add 2 ml of hexane and continue evaporating the ether–hexane solution to 0.3 ml.

Table I. Electron-Capture Detector Sensitivities,[a] Limits of Detection and Recovery Data[b] for Ten Halo- and Nitrophenol Ethyl Ethers

		Limits of	
Compound	Sensitivity, ng	Detection, ppm	Recovery, %
2,4-DCP	0.2	0.1	87–96
2,4,5-TCP	0.02	0.01	85–95
3,5,6-TCPyridinol	0.05	0.01	91–97
3,6-DCIP	0.02	0.01	88–94
2,5-DCBrP	0.02	0.01	88–96
PCP	0.01	0.01	92–96
PNP	0.1	0.02	85–98
PNC	0.3	0.05	88–98
2-C-4NP	0.05	0.01	85–92
DNOC	0.1	0.05	86–96

[a] Based on 15% scale deflection. [b] Based on use of the described method.

Prepare a silica gel chromatographic column as previously described. Prewash the column with 10 ml of hexane and discard the washing. Transfer the concentrated urine extract quantitatively to the column using 2 ml of the 20% benzene-in-hexane. As soon as the solvent sinks in the sodium sulfate, add 8 ml of the 20% benzene-in-hexane to the column and collect the total volume of the 20% benzene-in-hexane (10 ml). This fraction contains the halogenated phenols. Continue eluting with 10 ml of 40% benzene–hexane and discard this fraction. Add 10 ml of 60% benzene–hexane, followed by 10 ml of 80% benzene–hexane and collect these fractions in a single tube. The combined 60–80% benzene–hexane fractions will contain the nitrophenols. Add 10 ml of benzene to column and collect eluate. Frequently a small amount of the DNOC is found in the benzene fraction. The urinary impurities eluted by the benzene solvent do not interfere with the gas chromatographic determination of DNOC, which has a relatively long retention time. The elution pattern of spiked control urine extracts must be established before the analysis of actual samples is undertaken.

RESULTS AND DISCUSSION

Control rat urine samples were fortified with the sodium salts of the phenols. The samples were analyzed and aliquots of the 20% B-H and 60 + 80% fractions were injected separately into the gas chromatograph. Figure 1 illustrates chromatograms of the 20% B-H fraction (halogenated phenols) of spiked and control rat urine samples, and Figure 2 shows chromatograms of the 60 + 80% fraction (nitrophenols) of control and fortified rat urine samples. An average of 0.01 ppm of pentachlorophenol was routinely found in all control urine samples. The percent recovery, limits of detection in ppm, and detector sensitivity in nanograms (based on 15% scale deflection) are shown in Table I.

Ethylation of the phenols proceeds rapidly at room temperature, producing ethyl ether derivatives which are gas chromatographable and stable to silica gel column chromatography. Better gas chromatographic resolution of the more volatile phenols was achieved by preparing the ethyl

Table II. The Relation between Total Dosage of Biodegradable Pesticides and Urinary Excretion of Halo- and Nitrophenols

			Excretion of phenolic-type metabolites			
Compound	Dose level LD_{50}	nmol fed	Metabolite	nmol excreted	% of dose excreted	Days for complete excretion
VC-13	10^{-2}	5140	2,4-DCP	3470	67	3
	10^{-3}	514		359	70	1
Ronnel	10^{-4}	234	2,4,5-TCP	124	53	2
	10^{-5}	23.4		7.4	32	1
Dursban	10^{-3}	232	3,5,6-TCPyridinol	157	68	4
	10^{-4}	23.2		29.5	100+	4
C-9491	10^{-4}	314	3,6-DCIP	7.6	2.4	2
	10^{-5}	31.4		0.834	2.7	1
Bromophos	10^{-4}	617	2,5-DCBrP	305	49	3
	10^{-5}	61.7		41.4	67	3
PCP	10^{-4}	40.6	PCP	9.03	22	2
	10^{-5}	4.06		1.05	26	1
EPN	10^{-2}	670	PNP	108	16	3
	10^{-3}	67.0		9.35	14	3
Fenitrothion	10^{-2}	5430	PNC	3610	66	2
	10^{-3}	543		472	87	1
Dicapthon	10^{-2}	8700	2-C-4NP	3570	41	1
	10^{-3}	810		20	2.5	1
DNOC	10^{-2}	910	DNOC	0	0	
	10^{-3}	91.0		0	0	

derivatives instead of the more commonly used methyl ethers.

Silica gel column chromatography serves two purposes: it provides a clean sample for gas chromatographic analysis and it conveniently separates the halogenated phenols, thus simplifying the gas chromatographic analysis.

A mixture of the ten phenols and three phenoxy acids, namely 2,4-D, 2,4,5-T, and silvex, can be determined in one sample. All of the halogenated phenols involved in this study are eluted with 20% benzene–hexane, while the nitrophenols and the phenoxy acids are eluted in the 60 and 80% benzene–hexane fractions. These herbicides are included in this report because they are detected as intact excreted residues in the urine using the present procedure. 2,4-DCP and 2,4,5-TCP, potential mammalian metabolites of 2,4-D and 2,4,5-T, are also determined in this procedure. The analysis of these herbicides and their metabolites has been described in detail in a previous report (Shafik *et al.*, 1971a). In the gas chromatographic step, the retention time of the ethyl ether derivative of 2-chloro-4-nitrophenol is almost identical to that of the ethyl ester of 2,4-D. In addition, these two derivatives are eluted in the 60 + 80% benzene–hexane fractions from silica gel. Confirmation of identity can be accomplished using the classical $NaHCO_3$ and acid extraction steps which separate carboxylic acids from phenols (Bakke and Scheline, 1969).

In order to determine if a correlation exists between exposure to intact pesticides and excretion of urinary phenolic metabolites, male Charles River rats weighing 190 to 220 g were dosed by gavage with peanut oil solutions of eight organophosphorus compounds, PCP, and DNOC in concentrations ranging from 10^{-2} through 10^{-5} of the LD_{50} (Kenaga and Allison, 1969), as indicated in Table II. The doses were administered daily for 3 days to two rats at each dose level. The animals were maintained in stainless steel metabolism cages with the two rats administered the same dose regimen maintained in the same cage. Urine samples were collected at 24-hr intervals and stored in a freezer until analysis was performed.

Urine samples were analyzed for several days following the third dose until no detectable levels of the phenolic type metabolites were observed. This established the number of days required for total excretion of the metabolites. The percent of the total dose excreted as the phenolic metabolite was calculated from the sum of the amount of phenolic metabolite excreted each day and the total amount of pesticide fed in the 3-day period.

As indicated in Table II, the amount of urinary metabolites excreted is proportional to the dose of parent compound administered. The percent of the dose excreted in the urine as a phenolic metabolite of the pesticide fed indicates that low level animal exposure to VC-13, ronnel, Dursban, Bromophos, PCP, EPN, and fenitrothion can be detected. The low excretion rates of the phenols of C-9491 and dicapthon in urine indicate that the method cannot detect low-level exposure to these compounds. DNOC was not detected in the urine of rats fed 10^{-2} and 10^{-3} of the LD_{50} of DNOC.

There are other pesticide chemicals not included in this investigation which may produce the same phenolic metabolites reported in this study. 2,4,5-TCP can be a urinary metabolite of ronnel, Gardona, lindane, 2,4,5-T, and Silvex. PNP in urine can result from exposure to EPN, ethyl parathion, and methyl parathion. The method is not capable of distinguishing between the sources of the urinary 2,4,5-TCP and PNP. When such a distinction is essential, the corresponding alkyl phosphate of the organophosphorous pesticide can be determined in the urine (Shafik *et al.*, 1971a). The free phenoxy acids of 2,4,5-T and Silvex can also be determined in the urine (Shafik *et al.*, 1971a). It must be emphasized that the rat-feeding experiment was designed only for the purpose of evaluating the method and not as a study of the metabolism of these compounds.

In conclusion, a multiresidue method has been developed for the determination of low levels of halo- and nitrophenols and phenoxy acids in rat urine which undoubtedly can be extended to environmental samples. Such a method should be useful, if properly evaluated on a monitoring and surveillance scale, in establishing human exposure to low levels of a large number of biodegradable pesticides.

LITERATURE CITED

Bakke, O. M., Scheline, R. R., *Anal. Biochem.* **27**, 451 (1969).

Bradway, D., Shafik, M. T., "A Gas Chromatographic Method for the Determination of Low Levels of *p*-Nitrophenol in Human and Animal Urine," presented at the 162nd National Meeting of the American Chemical Society, Washington, D. C., September 12–17, 1971.

Kenaga, E. E., Allison, W. E., reprinted from *Bull. Entomol. Soc. Amer.* **15,** 85 (1969).
Menzie, C. M., Metabolism of Pesticides, Bureau of Sport Fisheries & Wildlife, Special Scientific Report, Wildlife No. 127, 1969.
Shafik, M. T., Sullivan, H. C., Enos, H. F., *J. Environ. Anal. Chem.* **1,** 23 (1971a).
Shafik, M. T., Bradway, D., Enos, H. F., *J. Agr. Food Chem.*, **19,** 885 (1971b).
Stanley, C. W., *J. Agr. Food Chem.* **14,** 321 (1966).

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20

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Determination of DDT and Related Substances in Human Fat

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Preliminary evidence of the occurrence of DDE as well as DDT in human fat made it necessary to study the Schechter-Haller method critically as applied to fat containing DDT and the degradation product, DDE. Through empirical standardization it was possible to make differential determinations of DDT and DDE totaling 5 micrograms in unknown fat samples, with rough estimations down to 2 micrograms. A modified Davidow column was used in a rapid method for isolating DDT and DDE. Among 50 samples all but 2 contained substantial quantities of DDE. The total DDT plus DDE ranged from 0 to 80 p.p.m., with DDE representing from 39 to 86% of the total. Chromatographic and spectrophotometric data provided evidence that the degradation product is DDE.

IN THE course of a study on the occurrence of DDT in human fat it became clear that a detailed study of both the method of isolating DDT from fat and the final estimation by the Schechter-Haller procedure was necessary. This became more essential when evidence was obtained that a substantial quantity of DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene] as well as DDT was present in the samples. A preliminary report on this observation has been given by Pearce *et al.* (*8*). The present paper reports the results of the study on the methods employed and some additional evidence as to the presence of DDE in human fat.

SEPARATION OF FAT

The isolation of DDT and related compounds from biological materials invariably has presented difficulties, especially in dealing with fats or oils because of the pronounced solubility of chlorinated hydrocarbons in fat solvents. The most commonly used methods for separating DDT from fat are based on a sulfuric acid treatment which partially sulfonates the fat and greatly reduces its affinity for DDT and thus permits extraction of the DDT in an inert solvent such as chloroform or carbon tetrachloride. In the method of Schechter *et al.* (*10*), the operation is carried out in separatory funnels. Davidow (*3*) has adapted the sulfuric acid treatment and solvent extraction to a chromatographic column. With modifications, the Davidow column has been found to be more rapid and considerably less laborious in the writers' experience. In the present work as well as in that already reported, the modified Davidow column has been employed. The fat samples were extracted in preparation for chromatographing as follows:

Samples of known weight were ground in a mortar with anhydrous sodium sulfate and then transferred to 500-ml. glass-stoppered bottles using C.P. carbon tetrachloride as solvent and transfer agent (50 to 70 ml. of carbon tetrachloride per 2 grams of fat). The bottles were shaken mechanically for at least 2 hours. The contents were then filtered and the filtrate was chromatographed through previously prepared Davidow columns (*2*).

SCHECHTER-HALLER ANALYSIS

The Schechter-Haller (*11*) method for determining DDT in micro quantities is now generally accepted as the best available. Various modifications have been introduced from time to time and it is doubtful whether any two laboratories follow exactly the same procedure. Application to analysis of spray residues, where relatively large amounts of DDT are involved, has in general been successful; however, in the case of fats and tissues and other biological materials containing relatively small amounts of DDT, considerable trouble has been experienced in the application of the method. In this work the size of the samples collected was usually small (1 to 3 grams) and in most instances the amounts of DDT were of the order of 5 to 10 p.p.m. Thus, reasonably accurate measurement down to and below 5 micrograms was desired. The magnitude and variation in blank values are of great importance with these low amounts of DDT and very careful empirical standardization of the method becomes necessary to maintain variation in duplicate analyses substantially below 10%. Moreover, application of the two-color equation of Knudson (*5*) is very unreliable unless light absorption due to blank is minimized and controlled. For these reasons preliminary factors affecting reagent blanks and blanks arising from use of the Davidow column have been carefully evaluated and the method has been modified accordingly.

FACTORS AFFECTING BLANKS

One cause of erratic and undesirably high blank values is the failure to remove all of the solvent before nitration. The state of apparent dryness of a particular sample is usually judged by odor and appearance, but the acuity of this judgment may vary from day to day for the same analyst and also between analysts. This variation can be prevented by adding a period of heating and aeration beyond the point of apparent dryness. It also has been found, as observed by Clifford (*1*), that heating the nitrated product just prior to solution in benzene and adding methylate to develop the color will help to maintain low and consistent blank values. The writers have adopted a prenitration heating time of 15 minutes at about 75° C. with simultaneous gentle aeration and a postnitration heating period of 30 minutes at 75° C. Using a 1-cm. absorption cell in a Beckman Model B Spectrophotometer, average reagent blanks at 520 and 597 mμ were found to be 0.007 and 0.004, expressed as optical density for a final volume of 6 ml.

It also became evident that some blank effect was arising from the Davidow column. Blanks occurring from elution of carbon tetrachloride through the column have been evaluated and found to average 0.007 and 0.005 net at 520 and 597 mμ, respectively. Thus, the combined column blanks and the Schechter-Haller reagent blanks averaged 0.014 and 0.009. These values appear to be the average minimum blank values attainable by the procedure used by the writers. In terms of *p,p'*-DDE and *p,p'*-DDT these values represent about 1.5 and 1.0 micrograms, respectively. If one is attempting estimation of 5 micrograms of total DDT and DDE, the blank values are obviously of major significance and unless they are reasonably constant great errors will appear in the calculated amounts of DDT and DDE present. The average deviation from the mean blank values has amounted to 0.4 and 0.2 microgram of DDE and DDT, respectively. With this degree of deviation, there will be no excessive error, even though the blank represents a substantial quantity of DDE and DDT.

One source of unusually high and variable reagent blanks encountered in this laboratory appears worth mentioning. The practice of cleaning glassware with aromatic solvents, such as xylene, is extremely hazardous. The last traces of these solvents are very difficult to remove from glass and apparently carry through the nitration, resulting in brownish yellow colors which absorb strongly throughout the spectrum.

The exact evaluation of blanks arising from the fat itself has presented some difficulty. The amount of human or animal fat free of DDT or related compounds is extremely limited. With the experimental material available it was found that the fat blanks were low and reasonably constant from about 500 through 600 mμ. This seems to agree with the experience of Offner and Calvary (*7*), who made an intensive study of this problem in connection with analysis of urine, liver, and other biological materials. Evidence that the fat blanks were low and substantially constant in the present study was obtained by analysis of composite fat samples, addition of known quantities of DDT and DDE to samples, and analysis after another method of fat elimination. In addition, several samples of human fat predating the introduction of DDT have been examined.

DETERMINATION OF DDT AND RELATED COMPOUNDS

Absorption spectra data (*8*) indicated that a substantial portion of the Schechter-Haller-positive compounds isolated from the human fat samples being examined was DDE, and that *o,p'*-isomers were not present in abnormal amounts, as no pronounced absorption occurred at 597 mμ. It seems probable that the *o,p'* and *p,p'* isomers would appear in fat in the proportion they occur in technical DDT—i.e., 1 to 4 or 5, respectively (*4*). Although one can determine *o,p'* and *p,p'* isomers by the Schechter-Haller method by treating them as a two-component color system by the method of Knudson *et al.* (*5*), resolving more than two components such as mixtures of *o,p'*-DDT, *o,p'*-DDE, *p,p'*-DDT, and *p,p'*-DDE is not feasible. For this reason it has been necessary to simplify the analysis by treating the isolated products as consisting predominantly of *p,p'*-DDT and *p,p'*-DDE. This simplification represents a practical solution to the complex problem of analyzing materials containing mixtures of *o,p'*- and *p,p'*-DDT and their degradation products.

Table I. Extinction Coefficients for DDT and DDE

At Wave Length 597 Mμ, *A*		At Wave Length 520 Mμ, *B*	
K_1^A, *p,p'*-DDT	K_2^A, *p,p'*-DDE	K_1^B, *p,p'*-DDT	K_2^B, *p,p'*-DDE
0.067	0.005	0.027	0.070

EVALUATION OF EXTINCTION COEFFICIENTS

Table I presents the extinction coefficients for pure *p,p'*-DDT and DDE after processing by the Schechter-Haller method as adapted in this laboratory (*2*).

The values were calculated from the equation $K = \frac{D}{C}$

where K = extinction coefficient
D = optical density, 1-cm. absorption cells
C = micrograms per ml. of colored solution

Using the values in Table I in the Knudson equations:

C_1 (micrograms of *p,p'*-DDT per ml. of colored solution) = $1.1 - (14D_{597} - D_{520})$

and C_2 (micrograms of *p,p'*-DDE per ml. of colored solution) = $5.9 - (2.5D_{520} - D_{597})$

where D_{520} = optical density at 520 mμ
D_{597} = optical density at 597 mμ

Since a total volume of 6 ml. of colored solution was used (2

Table II. Analysis of Known Mixtures of *p,p'*-DDE and *p,p'*-DDT

Sample No.	Added, γ		Found, γ		
	DDE	DDT	DDE	DDT	DDE + DDT
1	10	0	10.3	0.2	10.5
2	9	1	8.8	0.7	9.5
3	8	2	7.7	1.8	9.5
4	6	4	7.1	4.9	12.0
5	4	6	4.4	6.6	11.0
6	2	8	1.9	7.9	9.8
7	20	0	20.0	0.0	20.0
8	0	20	0.0	21.5	21.5
9	16	4	17.0	4.1	21.1
10	12	8	12.0	8.1	20.1
11	8	8	7.3	7.4	14.7
12	8	12	8.5	11.8	20.3
13	4	16	4.0	16.6	20.6

ml. of benzene plus 4 ml. of sodium methylate solution), the final equations as used were:

$$C_1 \text{ (micrograms of } p,p'\text{-DDT per 6 ml. of colored solution)} = 6.6\,(14D_{597} - D_{636}) \quad (1)$$

$$C_2 \text{ (micrograms of } p,p'\text{-DDE per 6 ml. of colored solution)} = 35.4\,(2.5D_{636} - D_{597}) \quad (2)$$

Known mixtures of the two components were run through the method in the absence of fat to test the reliability of these equations. Table II presents typical results of the analysis of various mixtures of the components.

The data indicate satisfactory resolution of *p,p'*-DDT and *p,p'*-DDE and are believed to represent the normal variation that might be expected in using the Schechter-Haller method for analysis of a two-color system under more or less ideal conditions.

Table III. Retention of *p,p'*-DDA, *p,p'*-DBP, *p,p'*-DDE, and *p,p'*-DDT by Davidow Column

Material Added (50 γ)	Found, γ	Recovery, %
p,p'-DDA	1.1	2.2
p,p'-DBP	4.0	8.0
p,p'-DDE	50.5	101.0
p,p'-DDT	48.7	97.0
p,p'-DDT[a]	50.6	101.2

[a] Not run through column.

BEHAVIOR OF DAVIDOW COLUMN

As the Davidow chromatographic column was being used to separate the fat, it was believed essential to determine whether it would retain DDE or other degradation products of DDT. Some representative tests are shown in Table III.

It is apparent that *p,p*-DDA [bis(*p*-chlorophenyl)acetic acid] and *p,p'*-DBP (4,4'-dichlorobenzophenone) are largely retained by the column, whereas DDE and, of course, DDT are passed. This behavior is further justification for basing calculations on only *p,p'*-DDE and *p,p'*-DDT.

Although there is no reason to suspect that the Davidow column causes any significant degradation of DDT, nevertheless, experiments were conducted to demonstrate this point. A few samples of human and monkey fat were available which were apparently free of DDT and DDE as determined by prior analysis. Known quantities of *p,p'*-DDT were added to the samples and then they were processed through the complete procedure. Good recoveries of DDT were obtained in all cases. This evidence, plus the many knowns which have been run through the columns in the absence of fat from time to time, leaves little doubt that the Davidow column causes no significant degradation of DDT.

TESTS OF METHOD AS APPLIED TO FAT

In order to test further the accuracy and reliability of the procedure adopted, known quantities of *p,p'*-DDE and *p,p'*-DDT were added to aliquots of eluates of human fat samples. Typical results are presented in Table IV. Samples 3, 6, and 9 represent standards containing the same amount of DDE and DDT as that added to the respective fat eluates which were analysed simultaneously with the eluates. Comparison of the figures showing micrograms recovered with the data on the standards as determined by analysis shows very good agreement. In other experiments one half of each of several fat samples was analysed separately. The remaining halves were combined in a composite sample and also analysed. Data on such experiments have been reported (*8*). These data as well as those in Table IV indicate the accuracy of the procedure adopted and also show that the blanks are essentially constant and being correctly evaluated.

Laug *et al.* (*6*) have published DDT analyses of human fat samples using the method of Prickett *et al.* (*9*), which involves an alkaline saponification to remove the interfering fat. This saponification dehydrohalogenates the DDT to DDE, which is estimated as such by the Schechter-Haller method and then calculated as DDT. The method, of course, fails to differentiate between any DDE present in the fat before dehydrohalogenation and that arising from dehydrohalogenation of the DDT present. It was of interest to compare this method of analysis with the one employed in the present study.

Eluates of two different samples were analysed by the writers' procedure and also after treatment with alcoholic sodium hydroxide according to the method of Prickett *et al.* (*9*).

One sample consisted of 15 grams of human fat, which was extracted with carbon tetrachloride and then passed through Davidow columns in five portions. The eluates were combined and the volume was reduced to 60 ml. Two 25-ml. aliquots (1 and 2, Table V), each representing 6.1 grams of fat, were used for analysis before and after dehydrohalogenation. The second sample was obtained by extracting and chromatographing a number of small fat samples containing from 2 to 20 p.p.m. of DDT and combining the eluates into a composite sample. Equal aliquots (3 and 4, Table V) of this composite solution representing 5.8 grams of fat were used for the analysis before and after dehydrohalogenation. All calculations were based on the two-color equations given earlier.

Table IV. Recovery of *p,p'*-DDE and *p,p'*-DDT Added to Human Fat Sample Eluates

Sample	Found, γ DDE	Found, γ DDT	Found, γ DDE + DDT	DDE and DDT, P.P.M.
1. Human fat A + 10 micrograms DDE and 5 micrograms DDT	13.3	9.7	23.0	..
2. Human fat A alone	2.9	4.3	7.2	6
Recovery of added DDE and DDT	10.4	5.4	15.8	
3. DDE and DDT alone by analysis	10.4	5.8	16.2	
4. Human fat B + 5 micrograms DDE and 5 micrograms DDT	11.7	11.9	23.6	..
5. Human fat B alone	5.4	7.7	13.1	10
Recovery of added DDE and DDT	6.3	4.2	10.5	
6. DDE and DDT alone by analysis	5.3	5.5	10.8	
7. Human fat C + 5 micrograms DDE and 5 micrograms DDT	13.2	11.5	24.7	..
8. Human fat C alone	7.9	6.3	14.2	3
Recovery of added DDE and DDT	5.3	5.2	10.5	
9. DDE and DDT alone by analysis	5.3	5.5	10.8	

Table V. Comparative Results of DDT-DDE Analyses before and after Dehydrohalogenation

	Micrograms DDE	Micrograms DDT	Micrograms Total as DDT	P.P.M.[a] DDE	P.P.M.[a] DDT	P.P.M.[a] Total as DDT	% DDE of Total DDE and DDT Originally Present
Single human fat sample							
1. Before	31.9	19.5	55.0	5.2	3.2	9.0	62
2. After	51.8	0.0	57.6	...	...	9.4	
Composite of human fat samples							
3. Before	22.4	15.9	40.8	3.9	2.7	7.0	59
4. After	36.3	0.0	40.3	...	...	6.9	

[a] 6.1 gram of fat for each of samples 1 and 2.
5.8 gram of fat for each of samples 3 and 4.

Table V shows that the total quantity of DDE and DDT calculated as DDT agrees extremely well both before and after dehydrohalogenation. This indicates that the method used does evaluate the amount of DDT present, as it is recovered quantitatively as DDE. Furthermore, it helps to substantiate that the component being measured as DDE is indeed DDE or other degradation products of DDT having similar absorption spectra.

SUMMARY OF HUMAN FAT ANALYSES

Table VI presents a summary of the analysis of 50 human fat samples examined by the procedure adopted (*2*). The samples were taken from different geographical areas and different occupational groups and are therefore not representative of any single group. Ninety-six per cent of the samples show detectable amounts of DDT and DDE. In no case was DDT found alone; DDE was always indicated as being present, to the extent of 39 to 86%. Some of these samples were obtained from individuals having known high exposure to DDT. It seems significant that even in these cases the materials isolated contained a high proportion of DDE.

In addition to the 50 samples reported in Table VI, 53 samples have been analyzed using a less refined analytical procedure. In the latter group all but four samples contained Schechter-Haller–positive materials and the average calculated DDE content was about 60% with a range of 40 to 90%. Among these samples one of particular interest was obtained from an individual who worked in a DDT formulation plant. The sample showed 122 p.p.m. of DDT and 127 p.p.m. of DDE. This suggests that DDT can be degraded to DDE in the human body, as it is not likely that this individual was exposed to any significant quantities of DDE.

The percentages of DDE reported in Table VI are necessarily somewhat in error, as the values were calculated from equations based on *p,p'*-DDT and *p,p'*-DDE only. The Schechter-Haller–positive materials occurring in human fat undoubtedly arise from technical grade DDT which may contain 15 to 20% *o,p'*-DDT. The latter isomer shows fairly strong absorption at 520 mμ, the wave length used to measure principally the DDE. The presence of *o,p'*-DDT in the fat samples will affect the values for both DDE and DDT when Equations 1 and 2 are used. While it is extremely difficult to evaluate this effect exactly, use of two-color equations based on technical grade DDT and *p,p'*-DDE give an approximation of the magnitude of the effect. For this reason the following equations were derived and applied to some representative fat samples:

$$\text{Micrograms of } p,p'\text{-DDE per 6 ml. of colored solution} = 46.2\,(1.93D_{520} - D_{597}) \quad (3)$$

$$\text{Micrograms of technical DDT per 6 ml. of colored solution} = 7.7\,(14D_{597} - D_{520}) \quad (4)$$

where

D_{520} = optical density at 520 mμ

D_{597} = optical density at 597 mμ

Table VI. Human Fat Analyses

Range, DDT plus DDE, P.P.M.	No. of Samples	DDE Range, %
Not detectable	2	. . .
2–5	12	40–62
6–9	19	39–86
10–19	7	43–63
20–29	2	53–81
30–39	3	54–63
40–49	1	78
50–59	1	56
60–69	2	65–74
70–79	1	49
Total	50	

Table VII. Comparison of Two Methods of Calculations Applied to Typical Human Fat Analyses

	Calcd.[a] as *p,p'*-DDT and *p, p'*-DDE				Calcd.[b] as Tech. DDT and *p,p'*-DDE			
Sample No.	DDE, p.p.m.	DDT, p.p.m.	DDT + DDE, p.p.m.	DDE, %	DDE, p.p.m.	DDT, p.p.m.	DDE + DDT, p.p.m.	DDE, %
1	2.3	2.3	4.6	50	2.4	3.2	5.6	43
2	3.1	2.9	6.0	52	3.5	4.3	7.8	45
3	6.3	6.7	13.0	48	6.7	9.7	16.4	41
4	4.6	3.9	8.5	54	5.2	5.6	10.8	48
5	11.4	6.8	18.2	63	13.2	9.9	23.1	57
6	2.9	2.3	5.2	56	3.2	3.2	6.4	50

[a] Equations 1 and 2.
[b] Equations 3 and 4.

Table VIII. Chromatographic Behavior of Various Nitrated Materials in Alumina Column

Material	Theoretical[a], γ	Solvent	Recovery, γ	Recovery %
p,p'-DDE	72.0	Acetone	0.0	0
		95% acetone	58.8	81
p,p'-DDT	68.0	Acetone	67.0	99
		95% acetone	0.0	0
Tech. DDT	68.0	Acetone	66.7	98
		95% acetone	0.0	0
o,p-DDT	17.5	Acetone	17.0	97
		95% acetone	0.0	0

[a] By analysis before chromatography.

A comparison of the data obtained using Equations 3 and 4 and 1 and 2 is presented in Table VII. The two sets of equations were applied to the same optical density values observed for each sample, so that direct comparison is valid. It is noteworthy that there is only a relatively small change in the calculated parts per million of DDE, whereas the difference in parts per million of DDT is much more marked. The proportion of DDE percentagewise shows only a moderate difference. Thus, the use of *p,p'*-DDE as one of the primary standards appears to introduce no major error. In any case, it is likely that any *o,p'*-DDE would be included with the *p,p'*-DDE, since their absorption characteristics are similar at the measuring wave lengths employed. One may then consider that all DDE values reported in this work represent the total of both isomers.

The most important aspect of the data in Table VII is the appreciable difference in the total DDT plus DDE calculated by the two methods. This difference is largely due to the fact that the extinction coefficients for *p,p'*-DDT and the technical product used as standards are markedly different at 597 mμ—i.e., 0.067 and 0.058, respectively. The lower value for the technical product is due to the presence of the *o,p'* isomer and probably some non-Schechter-Haller–positive materials. At 520 mμ the presence of the *o,p'* isomer produces a slightly higher extinction coefficient in the case of the technical product, 0.030 as compared to 0.027 for the *p,p'*-DDT. The net effect of the differences in the extinction coefficients of the two standards at both wave lengths is partially compensatory. However, the difference at 597 mμ outweighs that at 520 and thus tends to increase the total DDT calculated.

It would seem from this brief discussion that unless one knows the relative proportions of the major isomers present in a sample, the correct selection of primary standards is not possible. This means then that an arbitrary selection must be made when dealing with materials contaminated with technical DDT of unknown composition as in the case of human fat. Perhaps the closest approximation of the total DDT and degradation products present in foods or animal tissues could be attained by use of *p,p'*-DDE and a mixture of *o,p'*- and *p,p'*-DDT in the ratio of 1 to 4 as primary standards. However, the writers believe it is most practical for routine purposes to use simply *p,p'*-DDE and *p,p'*-DDT. There is some hope

Table IX. Analyses of Three-Component Mixture Containing 17.5, 68, and 72 Micrograms of o,p'-DDT, p,p'-DDT, and p,p'-DDE

(Chromatography, alumina column)

Recovery before, γ		Solvent	Recovery after, γ			
o,p'[a] plus p,p'-DDT	p,p'-DDE		o,p'[a] plus p,p'-DDT	o,p'-DDT[b]	p,p'-DDT[b]	p,p'-DDE
86.6	77.8	Acetone	85.5	19.1	66.4	0.0
		95% acetone	0.0	0.0	0.0	41.3
Recovery, % of Theoretical						
101	108		100	109	98	57

[a] o,p' plus p,p'-DDT calculated as technical DDT in combination with p,p'-DDE.

[b] Two-component equations for mixtures of o,p'- plus p,p'-DDT applied to acetone eluate.

Table X. Resolution of Nitrated Schechter-Haller–Active Material from Animal and Human Fat Samples

(Chromatography, alumina column)

	Recovery before Resolution, γ		Solvent	Recovery after Resolution			
Type of Sample	p,p'-DDE	Tech. DDT		p,p-DDE, γ	Tech. DDT, γ	p,p'-DDE, %	Tech. DDT, %
Rat fat (DDT-fortified diet)	24.4	68.8	Acetone	0.0	68.8	0	100
			95% acetone	15.7	0.0	64	0
Human fat (composite sample)	54.4	42.2	Acetone	0.0	42.1[a]	0	100
			95% acetone	26.3	0.0	48	0

[a] DDT found to contain 16% o,p'-DDT and 84% p,p'-DDT calculated by two-component equation for mixtures of o,p'- and p,p'-DDT.

of alleviating this general problem by use of chromatographic separations. Some progress has been made along this line and is described below.

CHROMATOGRAPHIC EXPERIMENTS

The chromatographic separation of DDT and its degradation products has been investigated in this laboratory in connection with the present study as well as studies of the degradation of DDT by resistant houseflies. In general, satisfactory quantitative separations of p,p'-DDT and DDE as such have not been successful.

A sharp separation of the nitrated products has been attained using 3 to 5 grams of 80- to 200-mesh absorption alumina (Fisher Scientific Co.) in columns 15 mm. in outside diameter and acetone as solvent. The products obtained after nitration by the Schechter-Haller method were taken up in 2 to 3 ml. of acetone and introduced to the column, using several more milliliters of acetone to transfer quantitatively onto the alumina. Enough acetone was then added to the top of the column so that 25 ml. of eluate could be collected. An elution speed of 20 to 30 drops per minute was maintained by use of a stopcock on the column. The acetone elution will remove o,p'-DDT, p,p'-DDT, and technical DDT quantitatively, whereas p,p'-DDE is completely retained on the column.

This is illustrated by the data submitted in Table VIII.

Attempts to elute the DDE from the column have not been totally successful. Only partial recovery has been realized so far, using a great variety of solvents. Ninety-five per cent acetone–water has given the best results, but from 20 to 50% of the DDE is usually lost. This loss is not due to incomplete elution, as exhaustive elution with 95% acetone–water as well as with other solvents fails to give quantitative yield of the original nitrated DDE. Nevertheless, the separation of the DDE from the DDT is quantitative and valid, since no DDE appears in the first acetone eluate. Complete absorption spectra of the eluted materials after treatment with the methylate reagent show no evidence of contamination of the DDT fraction with DDE nor does the partially recovered DDE show evidence of anything other than DDE. In Table IX resolution of a mixture of o,p'-DDT, p,p'-DDT, and p,p'-DDE by the technique is illustrated.

The chromatographic technique developed has been applied to rat and human fat samples and some typical results are shown in Table X. The data have been calculated in terms of technical DDT and p,p'-DDE. The DDT appears quantitatively in the acetone eluates. A complete absorption spectrum of the Schechter-Haller color developed for the human fat acetone eluate was typical of that of technical DDT showing evidence of the presence of about 16% of o,p'-isomer. As in the case of knowns, the DDE was found in the 95% acetone eluates, although quantitative recovery was not realized. Complete absorption spectra of the recovered products coincided with that of pure p,p'-DDE. These chromatographic data provide confirmatory evidence that DDE is present in the human fat samples and eliminate possible confusion with the o,p' isomer.

FAT SAMPLES PREDATING DDT

The evidence presented here and elsewhere (*8*) that DDT and a degradation product, probably DDE, occur in human fat is based entirely on spectrophotometric analysis of the Schechter-Haller–positive materials isolated by means of the Davidow chromatographic column. There is no apparent reason to doubt the validity of this evidence, as the colors produced by the Schechter-Haller method are highly specific for DDT and closely related compounds. It is extremely unlikely that compounds native to human fat would give colors characteristic of DDT and its relatives. In support of this, samples of fat have been obtained from autopsy specimens collected prior to the advent of DDT. Analyses of three of these samples are presented in Table XI. No trace of Schechter-Haller colors could be detected. A very slight yellow color developed, which is typical of blanks. The absorption due to this yellow color has been calculated in terms of DDE and DDT in Table XI merely to show the order of magnitude of the fat blanks.

Table XI. Analyses of Human Fat Samples Taken in 1938 and 1940

		Wave Length, Mμ		Equivalent of[b] Absorbance in Terms of			
		520	597				
Year Taken	Net Grams of Fat	Corrected[a] absorbance		p,p'-DDE, γ	p,p'-DDT, γ	p,p'-DDE, p.p.m.	p,p'-DDT, p.p.m.
1938	2.5	0.013	0.008	1.1	0.8	0.4	0.3
1940	1.9	0.004	0.008	0.1	0.9	0.1	0.5
1940	1.6	0.010	0.009	0.7	1.0	0.4	0.6

[a] Corrected for reagent and Davidow column blanks.

[b] No Schechter-Haller colors in evidence.

DISCUSSION

In the preliminary report (*8*) of this study analysis of individual samples and composite samples made up of the same samples was presented. The composite analyses agree very well with the sum of the individual analyses. These data have been supplemented in the present report by two additional treatments. First, known quantities of DDT and DDE have been added to fat samples and analyses made before and after such additions. The net DDE and DDT found by direct analysis and by subtraction of the known amounts added after analysis in their presence agree remarkably well (Table IV). Second, analysis by the writers' two-color treatment and by converting to DDE through dehydrohalogenation also shows excellent agreement (Table V). These three independent approaches would appear to be ample evidence that DDT plus a substantial proportion of the degradation product DDE is present in human fat. Supplementing these experiments are the data on the absorption spectra before and after dehydrohalogenation of the Schechter-Haller materials isolated (*8*). Many additional absorption spectra data have been collected by the writers, but have not been presented for the sake of brevity. These omitted data are in all cases confirmatory. It is also believed the chromatographic experiments are highly significant (Table X). Finally, the examination of fat samples predating the advent of DDT (Table XI) showed no evidence of the presence of Schechter-Haller–positive materials. The writers believe that DDT and DDE are contaminants of human fat of the general population.

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LITERATURE CITED

(1) Clifford, P. A., *J. Assoc. Offic. Agr. Chemists*, 30, 337–49 (1947).
(2) Communicable Disease Center, Technical Development Branch, Federal Security Agency, Savannah, Ga., Chemical Memorandum 1, 1952.
(3) Davidow, B., *J. Assoc. Offic. Agr. Chemists*, 33, 130–2 (1950).
(4) Haller, H. L., *et al.*, *J. Am. Chem. Soc.*, 67, 1591–602 (1945).
(5) Knudson, H. W., Meloche, W. V., and Juday, C., IND. ENG. CHEM., ANAL. ED., 12, 715–18 (1940).
(6) Laug, E. P., Kunze, F. M., and Prickett, C. S., *Arch. Ind. Hyg. and Occupational Med.*, 3, 245–6 (1951).
(7) Offner, R. R., and Calvary, H. O., *J. Pharmacol. Exptl. Therap.*, 85, 363–70 (1945).
(8) Pearce, G. W., Mattson, A. M., and Hayes, W. J., *Science*, 116, 254–6 (1952).
(9) Prickett, C. S., Kunze, F. M., and Laug, E. P., *J. Assoc. Offic. Agr. Chemists*, 33, 880–6 (1950).
(10) Schechter, M. S., Pogorelskin, M. A., and Haller, H. L., IND. ENG. CHEM., ANAL. ED., 19, 51–3 (1947).
(11) Schechter, M. S., Soloway, S. B., Hayes, R. A., and Haller, H. L., *Ibid.*, 17, 704–9 (1945).

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Recent applications of mass spectrometry and combined gas chromatography-mass spectrometry to pesticide residue analysis

BY

FRANCIS J. BIROS*

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I. Introduction

The value of mass spectrometry as a means of qualitative analysis of unknown compounds or for confirmation of the identity of sus-

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pected compounds is well recognized. Mass spectra provide information concerning the structural arrangement of atoms within a molecule on the basis of the mode of fragmentation of the molecular ion which is usually produced by electron impact. Fragmentation patterns resulting from bond fission and rearrangement of atoms are highly diagnostic and characteristic of the original molecular structure. Mass spectral interpretations are usually made on the basis of empirical correlations or detailed ion decomposition mechanisms. Theories and principles governing the interpretation of mass spectra have been discussed in excellent texts (BEYNON 1960, McLAFFERTY 1963, BIEMANN 1962) and comprehensive reviews (COOKS 1969).

GUNTHER (1962) stated the potential utility of mass spectrometry in evaluating metabolic pathways of pesticidal chemicals, although at that time the technique had not been employed extensively in pesticide residue assays. Recently, several reviews have considered the role of mass spectrometry in chemical structure evaluations with special reference to pesticide residue analysis (KANTNER and MUMMA 1966, WESTLAKE and GUNTHER 1967). Noting that the mass spectrometric technique provides comparative identification of gas chromatographic peaks, and thus would be of interest to pesticide residue chemists, these authors attributed the lack of information regarding the application of the technique for pesticide residue analysis to unavailability of instrumentation, a previous general requirement of milligram-sized samples for analysis, and the need for highly skilled operators due to the sophistication of equipment. Developments in instrument technology during recent years, however, have substantially diminished these difficulties. The increasing magnitude of literature data regarding applications of mass spectrometry to analysis of pesticide residues attests to the fact that the instrumentation has evolved to a point where cost and access to equipment is no longer a critical factor. Additionally, improvements in instrumental sensitivity, fast scan capability, reliability, and ease of operation have increased the scope of applicability of mass spectrometry, both separately and combined with gas chromatography to encompass such areas as the analyses of pesticide residues where sample characteristics of type and quantity frequently require special analytical considerations.

Recent reports have focused attention on the need for confirming pesticide residues, particularly those isolated from environmental samples (ELGAR 1967, SCHECHTER 1968). Of the spectroscopic and chemical techniques available for confirmation of residues, the combined gas chromatographic-mass spectrometric approach affords many advantages including rapid analysis, elimination of the necessity for isolating minute quantities of pure samples, and greater certainty in identification of an eluted component than that achieved by gas chromatographic retention times alone.

Because of the definitive structural information obtained by mass spectrometry, proper use of the technique would obviate the necessity (WESTLAKE and GUNTHER 1967) for using two different carefully selected techniques to help characterize pesticide residues.

Correlations of mass spectral fragmentation pathways with the structures of pesticides are required to obtain background information for interpreting the mass spectra of unknown pesticide metabolites. Fortunately, the chemical modes of fragmentation of a large number of pesticidal compounds have been determined. This information is particularly useful in those instances when only microgram (μg.) quantities of materials are available for confirmation or structural characterization. Mass spectrometry offers the best approach in such cases because of its high structural information content and the relatively low sensitivity of such complementary techniques as nuclear magnetic resonance spectroscopy. The investigator may then rely on this single instrumental source of information for all the structural data when sample size is limited.

In view of current interest in the chemistry of photodecomposition of pesticide residues induced by ultraviolet radiation, especially with regard to the identity and toxicity of the degradation products, it is well to mention the existence of relationships between the unimolecular reactions of ions produced in the mass spectrometer and the photochemical behavior of certain classes of compounds. TURRO *et al.* (1965) have elaborated on the consequences of such an interrelationship for a number of small ring compounds including ketene dimers, benzylcyclopropane, and nortricyclanone. Thus, mass spectral fragmentation correlations may provide a useful tool for predicting and interpreting photochemical reactions. Such correlations have already been shown to exist in a number of specific instances.

By far the bulk of information related to the mass spectral behavior of pesticide residues involves the application of electron impact ionization techniques. This review will be limited to applications of mass spectrometry employing electron impact ionization techniques. Nevertheless, other techniques for producing ions in the ion source of a mass spectrometer are available, including chemical ionization and field ionization; these methods generally complement electron impact data. DAMICO *et al.* (1969) have compared field ionization and electron impact spectra of some pesticidal compounds. Generally, molecular ion intensities are enhanced and fragmentation processes somewhat less complex. Thus, field ionization may provide structural information about a complex organic molecule where electron impact data may leave some doubt with respect to the identity of the molecular ion and its subsequent decomposition paths. Certainly further study is indicated in the application of field ionization techniques. Similar characteristics of chemical ionization mass spectrometry have also been reported (FALES *et al.* 1969). The present report will attempt to outline available literature data on the application of mass spectrometry as an individual technique and also combined with gas chromatography, both to confirm pesticide residues of suspected structure and to analyze and characterize residues of unknown structure.[1] A limited discussion of the chemistry of electron impact-induced fragmentation of several classes of pesticides will also be presented. Some emphasis will be placed on the techniques useful for isolating individual pesticide residues from various human, animal, and environmental substrates, particularly those involving isolation and trapping of samples by separation techniques such as gas chromatography and thin-layer chromatography. Additionally, gas chromatograph coupling techniques and other instrumental considerations especially pertinent to mass spectrometer sensitivity will also be discussed.

II. The mass spectral fragmentation behavior of pesticides

a) *Organophosphorus pesticides*

CAVANAUGH (1963) examined the mass spectra of a number of organophosphorus pesticidal compounds including parathion, malathion, the thiono and thiolo isomers of Systox, Phosdrin, DDVP, and Guthion. Significant and unusual or unique ion fragments were reported for these compounds from m/e 30 to m/e 221. No attempt, however, was made to elucidate chemical modes of fragmentation under electron bombardment for the individual pesticides studied. More recently, detailed mass spectral studies have been performed on a series of organophosphorus compounds by DAMICO (1966), JORG *et al.* (1966), and COOKS and GERRARD (1968). The mass spectral fragmentation behavior of four classes of pesticide compounds were reported, including phosphates, phosphorothionates, phosphorothiolates, and phosphorodithioates.

Molecular ion peaks were observed for all pesticides except Delnav and phosphamidon. Compounds such as gutoxon and malaoxon exhibited weak molecular ion peaks, thus requiring a relatively large sample to detect them. Electron-impact induced rearrangements of thiono to thiolo isomers were also detected in the mass spectra of several phosphorothionate and phosphorodithioate insecticides. In general, intense peaks are produced for all classes of organophosphorus pesticides when alpha-cleavage occurs with the charge on the phosphorus atom:

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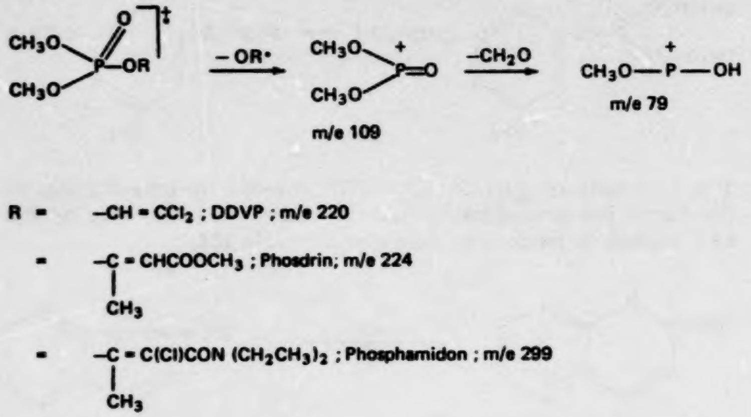
[1] Common, trade, and chemical names of the pesticides discussed in this article are presented in Table IV.

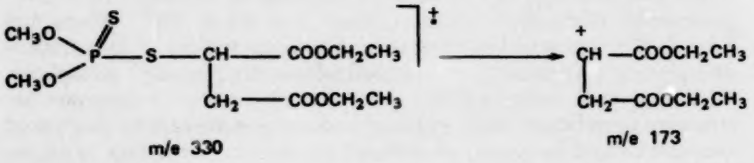


R = $-CH=CCl_2$; DDVP ; m/e 220

= $-\underset{\displaystyle CH_3}{\underset{|}{C}}=CHCOOCH_3$; Phosdrin; m/e 224

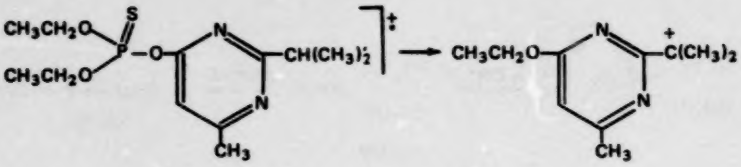
= $-\underset{\displaystyle CH_3}{\underset{|}{C}}=C(Cl)CON\,(CH_2CH_3)_2$; Phosphamidon ; m/e 299

Beta-cleavage, with the charge on the *R* group, was found to be of importance for the phosphorothiolates. The phosphorodithioates, illustrated by malathion, exhibited similar decompositions:

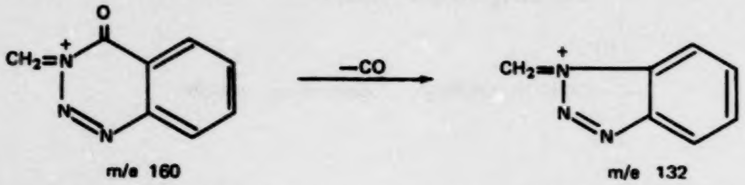


In addition the base peaks of Methyl Trithion (m/e 157, C_7H_6SCl), Imidan (m/e 160, $C_9H_6NO_2$), Guthion (m/e 160, $C_8H_6N_3O$), and Trithion (m/e 157, C_7H_6SCl) are likely formed by the same process.

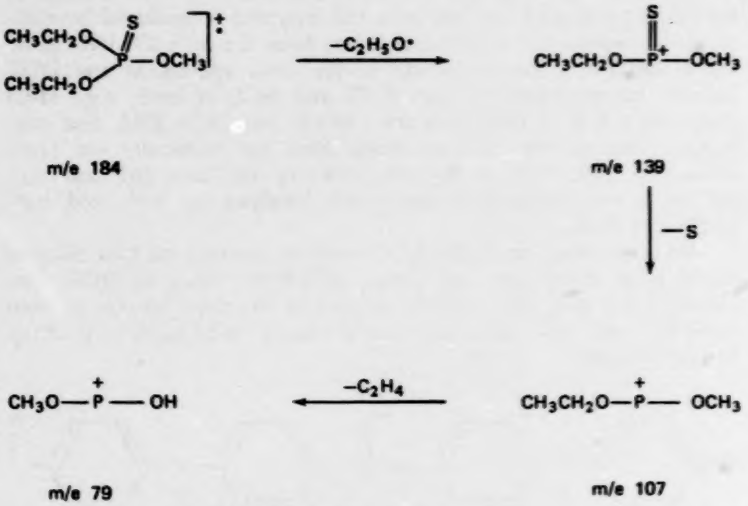
Ions due to rearrangement, such as those proposed by QUAYLE (1959), involving beta-bond fission with back transfer of hydrogen, were observed for ethion, Delnav, Di-Syston, and Trithion: (m/e 97, ($[(HO)_2PS]^+$). Similar Quayle-type rearrangements were observed for four phosphorothionates and two phosphates. Of significant contribution to the mass spectra of these organophosphorus compounds were fragment ions formed by rearrangements involving the complex alkoxy group (R). Thus, the base peak of Diazinon (m/e 179) could be formed by migration of an ethyl group from an ethoxy group to the oxygen atom of the R group:



The base peak of gutoxon (m/e 160), formed by beta-cleavage of the parent ion, undergoes further dissociation with loss of a neutral CO molecule to produce an intense peak at m/e 132:



The fragmentation behavior of certain trialkylphosphate and phosphonate compounds has been examined by Quayle (1959), McLafferty (1956), and Bafus *et al.* (1966). These compounds are of interest because they can be considered to be derivatized metabolic or hydrolytic products of organophosphorus pesticides. Because of the potential significance of these materials as indicators of organophosphorus pesticide exposure (Shafik and Enos 1970), Biros and Ross (1970) examined the low-resolution mass spectra of four trialkyl phosphates, two trialkyl phosphorothiolates, four trialkyl phosphorothionates, and three trialkyl phosphorodithioates. Fragmentation-structure correlations were outlined and some pathways to prominent ions established by means of metastable ion data. Important reactions for the phosphates include replacement of an ethyl group by a hydrogen atom. Secondary processes involve double rearrangements and fission of P-OR bonds, with the latter process predominating in the case of trimethyl-substituted phosphorus compounds. Similar fragmentation processes were observed for the phosphorothionates, in addition to internal electron impact-induced rearrangements of P(:S)-OR to P(:O)-SR of the type proposed by Jorg *et al.* (1966). Significant fragments due to abstraction of S atoms and SH radicals were also detected. Trialkyl phosphorodithioates undergo fragmentation processes involving combinations of those observed for the phosphorothionates and phosphorothiolates. These studies further showed (Shafik *et al.* 1970) that substantial differences in mass spectral fragmentation behavior can be expected from structural isomers within this group of organophosphorus compounds, e.g., those isomers produced by alkylation (with diazomethane or diazoethane) of diethylphosphorothioate. Thus, DEMMTP undergoes dissociation processes leading to formation of a m/e 79 ion ($CH_3O\overset{+}{P}OH$) and a m/e 107 ion ($C_2H_5O\overset{+}{P}OCH_3$) which are not observed for the phosphorothiolate (DEMMPTh) isomer:

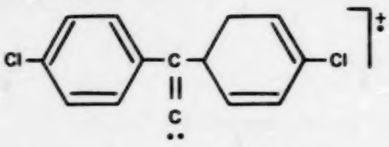


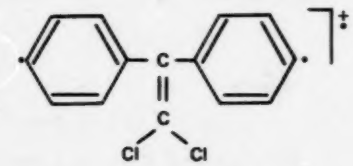
b) *Organochlorine compounds*

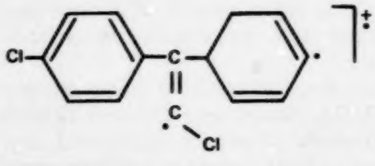
Mass spectral data for a number of halogenated pesticides including representative aromatic and cyclodiene type compounds have been reported by Mumma and Kantner (1966), who tabulated peak intensities and m/e values for important fragment ions without extensive discussions of fragmentation processes. Subsequently Kantner and Mumma (1966) tabulated additional data on a series of organochlorine pesticides and metabolites.

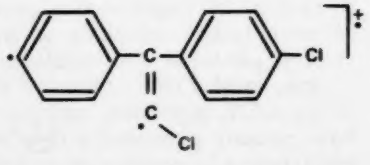
Jorg *et al.* (1966) discussed the chemical mode of fragmentation of *p,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDA. Sphon and Damico (1970) have recently presented a detailed study of eleven chlorinated aromatic pesticidal compounds including substituted diphenyl derivatives of methane, ethylene, and methanol. As expected, the most abundant ion in the mass spectra of six diphenylmethane pesticides including isomers of DDT and DDD, as well as Perthane and methoxychlor, was produced by cleavage of the bond beta to the aromatic rings with loss of a CCl_3 or $CHCl_2$ moiety. For *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDD, and *o,p'*-DDD, this process leads to the formation of a base peak of m/e 235 due to the generation of a dichlorodiphenylmethane-charged species or substituted phenyltropylium ion. Similar bond fission results in base peaks of m/e 223 and m/e 227 for Perthane and methoxychlor, respectively. Additionally, the mass spectra of these pesticides are characterized by the facile formation of an m/e 165, fluorenyl ion fragment, suggested by JORG *et al.* (1966), and verified by comparison of the low-voltage spectra of the isomers of DDT and DDD with 9-dichloromethylfluorene. Furthermore, metastable ion studies indicated that the m/e 165 fragment is produced by stepwise elimination of two chlorine atoms from the m/e 235 base peak. Other significant ion fragments in the mass spectra of the DDT isomers are produced by loss of Cl and HCl, or both, e.g., M-Cl (m/e 317), loss of HCl from the (M-Cl) ion (m/e 281), and consecutive loss of two chlorine atoms from the molecular ion (m/e 282). The difference in the intensities of the m/e 281 and m/e 282 peaks can be used to distinguish between the *o,p'*- and *p,p'*-isomers of DDT.

Abundant ions are formed by stepwise cleavage of two chlorine atoms from the parent ion (M+) of either isomer of DDE. The [M-2Cl] ion, m/e 246, is the base peak in the mass spectra of both *o,p'*-DDE and *p,p'*-DDE and was deduced to be composed of up to four isomers:



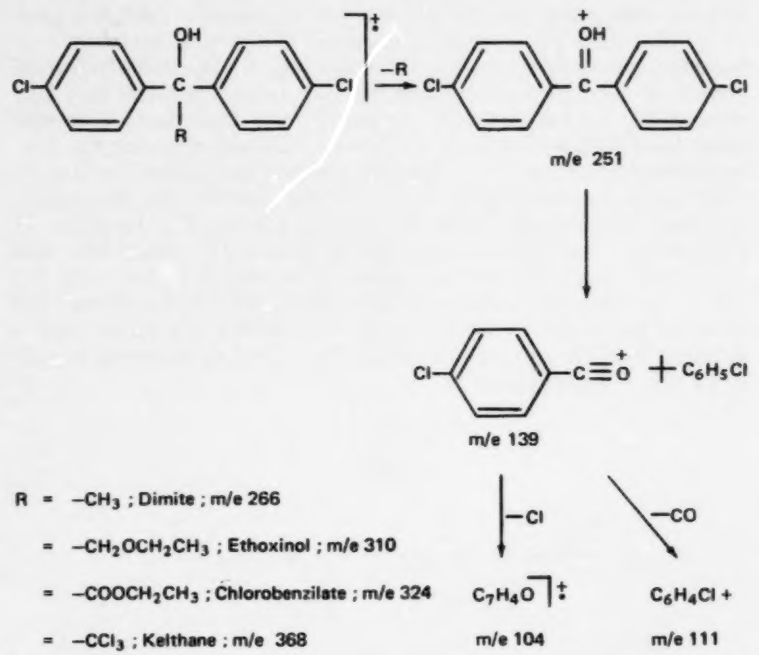






This deduction was based on mass spectral data obtained for 1,1-dichloro-2,2-bis(phenyl) ethylene and 1,1-dichloro-2,2-bis(*p*-bromophenyl) ethylene and metastable ratio values for *o,p'*-DDE, *p,p'*-DDE, and 9-dichloromethylenefluorene at different electron beam energies. Consecutive or simultaneous loss of two chlorine atoms from m/e 246 results in characteristically intense fragment peaks at m/e

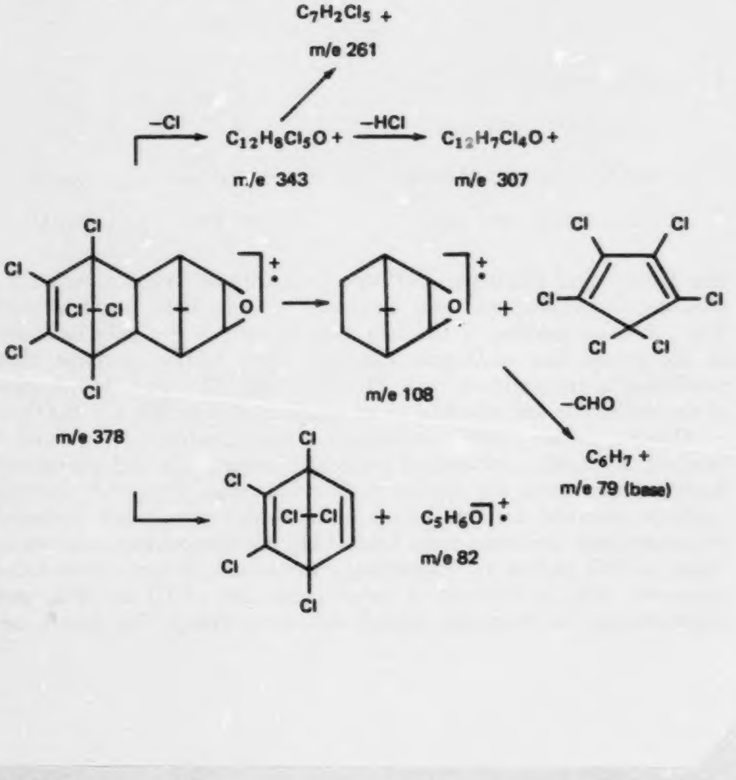
176 and m/e 211. Electron impact-induced fragmentation of pesticides with diphenylmethanol type structures is markedly influenced by the presence of the hydroxyl group. The following fragmentation pathway was observed for all diphenylmethanol pesticides examined:



R = $-CH_3$; Dimite ; m/e 266
= $-CH_2OCH_2CH_3$; Ethoxinol ; m/e 310
= $-COOCH_2CH_3$; Chlorobenzilate ; m/e 324
= $-CCl_3$; Kelthane ; m/e 368

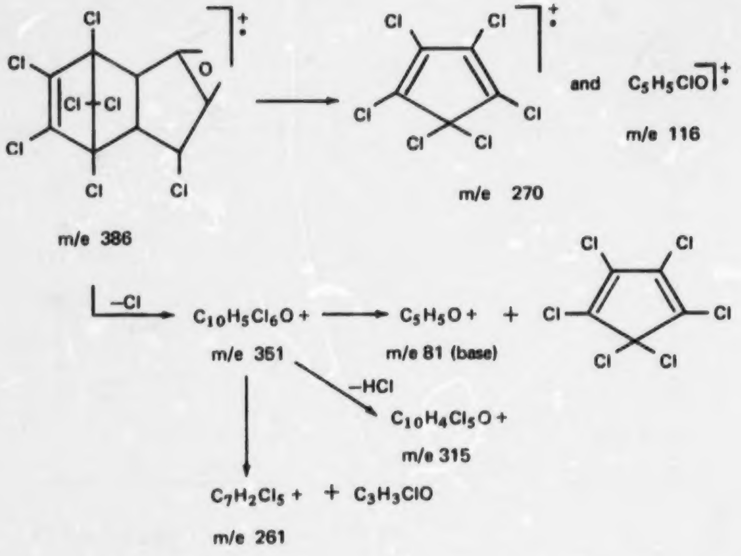
For Dimite and Ethoxinol, the base peaks are observed at m/e 251, whereas Chlorobenzilate and Kelthane produce base peaks at m/e 139. Of some interest is the fact that Dimite is the only pesticide of the group that undergoes cleavage alpha to the aromatic ring, producing a fragment of m/e 155, (M-C_6H_4 Cl), and dehydration of the molecular ion, resulting in an ion peak at m/e 248, (M-H_2O).

Damico *et al.* (1968) investigated the mass spectra of several bridged polycyclic chlorinated pesticidal compounds and postulated fragmentation paths for the more abundant ions. Important spectral features observed for this group of pesticidal compounds included fragmentations involving retro Diels-Alder decompositions, successive losses of HCl and/or Cl fragments, combination of retro Diels-Alder processes with preliminary or subsequent loss of Cl or HCl, and fragmentation involving the epoxide functional group. The dimethanonaphthalene derivatives produce intense peaks due to elimination of hexachlorocyclopentadiene as the neutral species in a retro Diels-Alder reaction. The charged fragment is m/e 92 for aldrin and isodrin and m/e 108 for the epoxy analogs, dieldrin and endrin. Aldrin and isodrin also undergo a decomposition in which either the hexachloronorbornadiene ion at m/e 296 or the cyclopentadiene ion at m/e 66 may carry the charge. Dieldrin and endrin exhibit a peak of m/e 82 which also suggests elimination of a neutral hexachloronorbornadiene molecule from the molecular ion. The spectra of all compounds of the dimethanonaphthalene type exhibit peaks at m/e 261, representing the ion $C_7H_2Cl_5^+$, suggesting retro Diels-Alder decomposition combined with loss of a Cl atom. Decomposition of the norbornadiene oxide ion (m/e 108) of dieldrin and endrin by loss of CHO yields an intense peak at m/e 79. For dieldrin this dissociation accounts for the base peak. The $C_5H_6O^{+\cdot}$ (m/e 82) fragment of both dieldrin and endrin loses CO to give $C_4H_6^{+\cdot}$ (m/e 54), and the $C_5H_5O^+$ (m/e 81) ion also loses CO to give a $C_4H_5^{+\cdot}$ (m/e 35) species. In these same spectra, peaks at m/e 315 for loss of Cl and CO from the parent peak are observed as a result of a two-stage reaction in which the Cl is presumed to be lost first. The fragmentation scheme of dieldrin is representative:

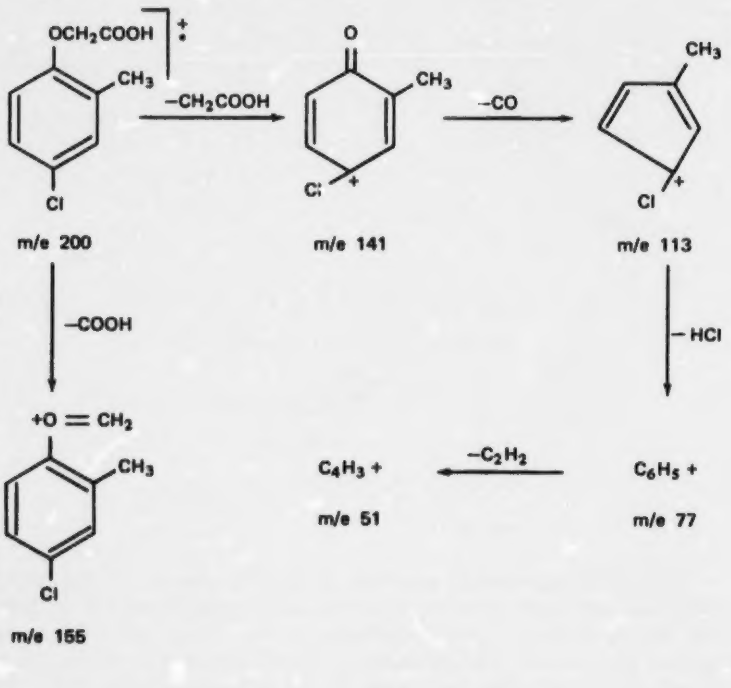


Compounds of the methanoindene type all give hexachlorocyclopentadiene ions at m/e 270 as probable retro Diels-Alder products. In the spectra of chlordene, heptachlor, and heptachlor epoxide, ions formed from the remaining portion of the molecule of m/e 66, m/e 100, and m/e 116, respectively, are produced. By contrast, chlordane and nonachlor preferentially tend toward having the charge located on the unsaturated moiety with which the observed presence of m/e 270 and absence of (M-270) ions are in accord.

The $C_7H_2Cl_5^+$ (m/e 261) fragment was observed in the spectra of heptachlor epoxide and chlordane. Furthermore, relatively intense isotope peaks at m/e 235, m/e 237, and m/e 239, verified by accurate mass measurements as a $C_5Cl_5^+$ species, were detected in the mass spectra of the methanoindene type pesticides. In the formation of this ion, a combination of loss of a chlorine atom and a retro Diels-Alder process is the most probable route. The fragmentation mode for the methanoindene type pesticides can be illustrated by the following dissociation scheme for heptachlor epoxide:



Typical fragmentation reactions of several classes of important pesticides and plant growth regulators have been discussed by Jorg and his co-workers (1966). Included in this study were the mass spectra of several chlorophenoxyacid-type herbicides. These compounds yield molecular ion peaks of substantial intensity and with characteristic chlorine isotope distribution peaks. Significant M-45 peaks arising from loss of COOH are observed for MCPA (m/e 155), MCPP (m/e 169), 2,4-D (m/e 175), and 2,4,5-T (m/e 209). Cleavage of the ether side chain occurring both alpha- and beta- to the ring are also important processes resulting in m/e 161 and m/e 145 peaks for 2,4-D, and m/e 141 and m/e 125 fragments for MCPP and MCPA. The m/e 125 ion for both compounds was regarded as a chlorotropylium fragment resulting from aryl rearrangement. Further decomposition due to consecutive loss of HCl (m/e 89) and acetylene (m/e 63), or alternatively, loss of acetylene (m/e 99) and HCl (m/e 63), was also demonstrated. Loss of chlorine atoms from the parent ion appeared to be important only for the pesticides substituted on the benzene ring with more than one chlorine atom such as 2,4-D, 2,4-DB, and 2,4,5-T. A hydroxytropylium ion (m/e 107), resulting from loss of chlorine from a fragment of ClO+ (m/e 142) composition was, however, observed for MCPA and MCPP, in addition to the above mentioned fragment formed by loss of HCl from the chlorotropylium ion. The following fragmentation scheme for MCPA is typical of this class of pesticide:

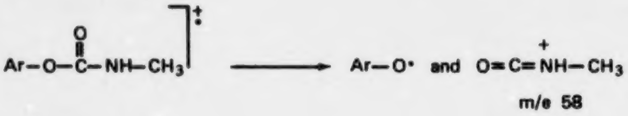


Utilizing high resolution instrumentation and photoplate recording of mass spectra, LOVINS (1969) has reported the identification of pesticides in homogeneous mixtures containing two to six compounds of the organochlorine and organophosphorus chemical types. Ions having exact masses unique for the spectra of each pesticide were chosen on the basis of abundance and type of fragment the ions corresponded to. These peaks were then used to identify the presence of the pesticide in the high resolution mass spectrum of a pesticide mixture. Mass spectra of 12 pesticides were tabulated and the potential utility of the method discussed in this preliminary study.

c) *Carbamate pesticides*

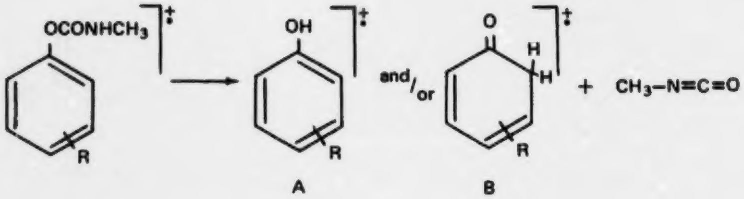
DAMICO and BENSON (1965), employing time-of-flight instrumentation, reported on the fragmentation characteristics of fourteen carbamate pesticidal compounds. Spectral data and detailed peak assignments were presented. Four aromatic *N,N*-dimethyl carbamates including Isolan, Dimetilan, Pyramat, and 1-naphthyl-*N,N*-dimethyl carbamate, had base peaks of m/e 72 in common, most likely representing the *N,N*-dimethylisocyanate ion formed by cleavage of the C—O bond beta to the aromatic ring. This fragmentation accounted for a significant portion of the total ion current for these pesticides presumably because of the stability of both the *N,N*-dimethylisocyanate ion and the aromatic radical formed by the cleavage.

Examination of the mass spectra of a number of *N*-methyl carbamates revealed a characteristic fragmentation process illustrated by the following equations:

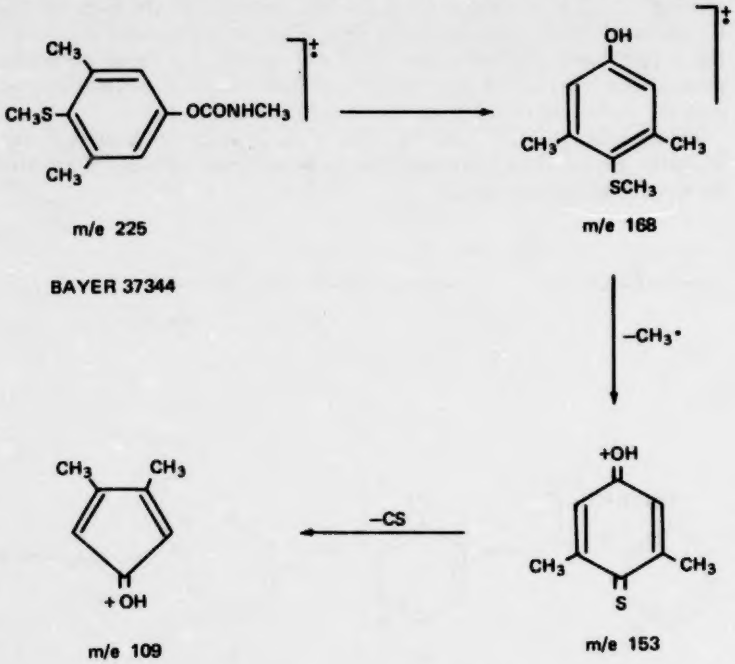


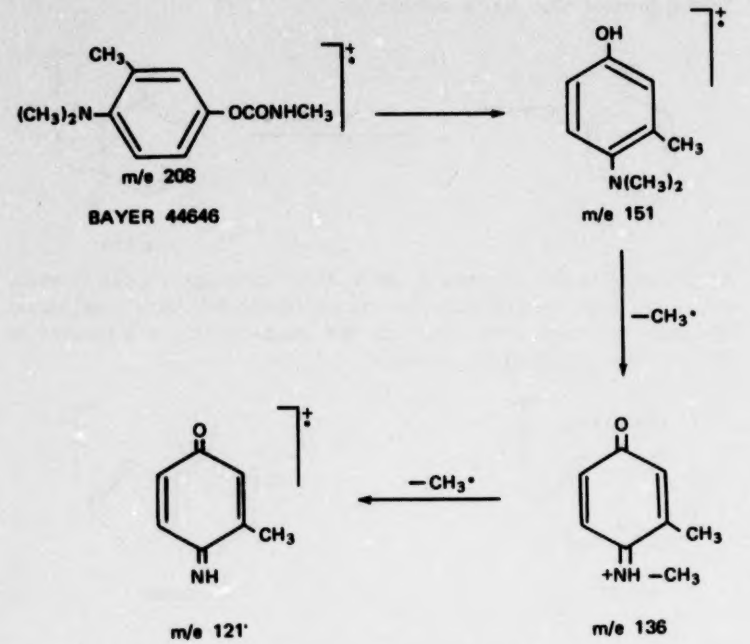
$$\left[\mathrm{Ar{-}O{-}\overset{\overset{O}{\|}}{C}{-}NH{-}CH_3}\right]^{+\cdot} \longrightarrow \mathrm{Ar{-}O\cdot}\ \text{and}\ \mathrm{O{=}C{=}\overset{+}{N}H{-}CH_3}$$

m/e 58



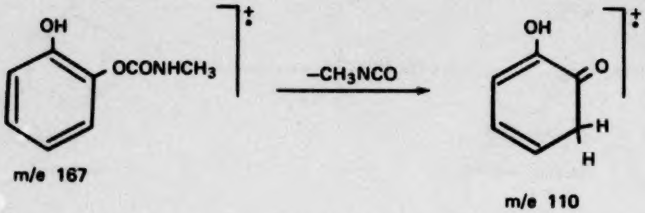
A significant contribution from the m/e 58 fragment was found in all the *N*-methyl carbamates studied and on the basis of the fact that the *N*-methyl-carbamate group was common to all pesticides studied in this class, the species $CH_3{-}NH{=}C{=}O^{+}$ was assigned to this fragment. A rearrangement ion corresponding in structure to either phenol A or ketone B was additionally detected in all members of this class. Evidence was detailed by the authors to show that hydrogen transfer to oxygen (to form phenol ion A) is probably the predominant mechanism. The remaining details of the mass spectra were characterized by processes related to the nature of the aromatically substituted R group as exemplified by Bayer 37344 below, and Bayer 44646 on p. 15. Also reported by DAMICO and BENSON were the mass spectra of IPC and CIPC, two *N*-phenyl carbamate pesticides. The base peak of these compounds was found to be m/e 43, corresponding to cleavage of the isopropyl group. Intense peaks were observed at m/e 93 for IPC and m/e 127 for CIPC corresponding to aryl amine rearrangements: $C_6H_5NH_2^+$ for IPC and $C_6H_4ClNH_2^+$ for CIPC. A fragment of appreciable intensity due to elimination of propylene (M-42) from IPC (m/e 137)and CIPC (m/e 171) was also observed. Although



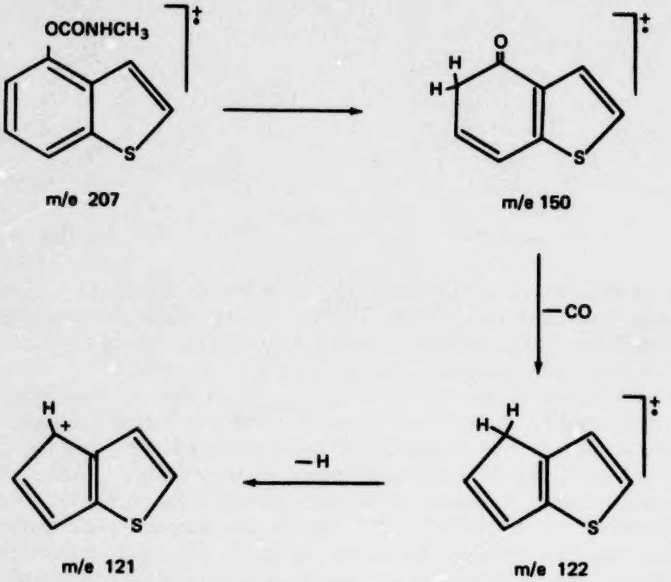


not specifically concerned with the fragmentation behavior of carbamate-type pesticides, Lewis (1964 a and b) earlier had discussed in detail the fragmentation behavior of similar *N*-phenyl carbamate compounds with proposed mechanisms based partially on the observed behavior of several deuterium analogs under electron impact.

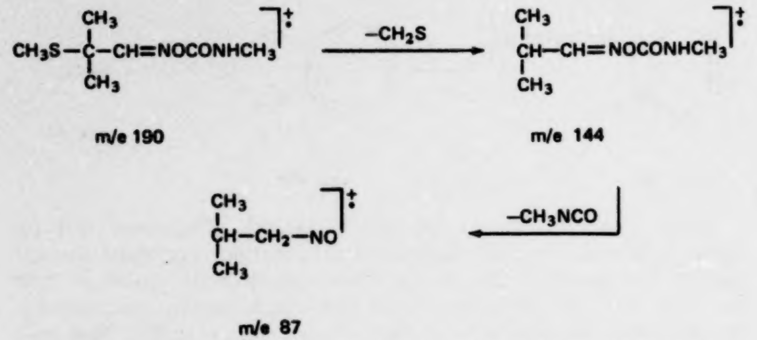
Subsequently, Benson and Damico (1968) extended their studies on the mass spectral properties of carbamates by reporting detailed research involving 28 compounds including representative carbamates, dithiocarbamates, thiocarbamates, and phenyl ureas. Included in this study were ten *N*-methyl carbamates whose fragmentation patterns closely paralleled those discussed above. A principal characteristic of the aryl-*N*-methyl carbamate spectra was the (M-57) ion resulting from the loss of a $CH_3—N=C=O$ molecule and concurrent formation of a phenol or substituted cyclohexadienone-type fragment ion involving either a four- or six-membered transition state rearrangement. No definitive evidence was adduced to identify conclusively the predominant process, but the mass spectra of model compounds argued against a four-membered transition state in at least one compound. Thus, the mass spectrum of catechol was markedly different from that of 2-hydroxyphenyl-*N*-methyl carbamate, indicating that the following process most likely occurs:



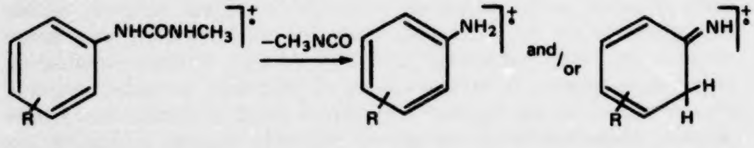
As discussed in the previous report by these investigators, the essential remaining mass spectral characteristics were derived from bond cleavage and rearrangements involving the aromatic ring substituents or the aromatic ring itself, for example:



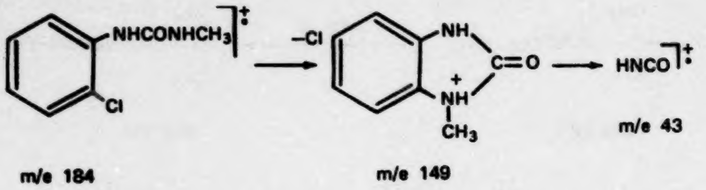
The two *N,N*-diphenyl carbamates studied exhibited fragmentation behavior similar to that observed for *N,N*-dimethyl carbamates. Thus, phenyl *N,N*-diphenyl carbamate (M^+, m/e 289) dissociates to form a base peak at m/e 196 corresponding to the *N,N*-diphenylisocyanate ion as the predominant process. Further bond fission results in a fragment of m/e 77 ($C_6H_5^+$) due to loss of phenyl isocyanate, and a fragment at m/e 168 (diphenylamine, $C_{12}H_{10}N$), resulting from expulsion of CO. A facile ring closure of the m/e 168 fragment with loss of a hydrogen atom was also observed. Temik and its derivative sulfoxide and sulfone compounds exhibited differences in mass spectral patterns from other *N*-methyl carbamates which were attributed to the greater probability of processes involving the sulfur atom to occur initially:



Thomson *et al.* (1966) studied the electron impact induced rearrangements of 12 carbamates and thiocarbamates in order to compare the possible elimination of CS, COS, or CS_2 with the well documented loss of CO and CO_2 observed in the mass spectra of other classes of compounds, particularly organic carbamates. In fact, these investigators found that rearrangement with elimination of the central portion of the molecule as CS, COS, or CS_2 was insignificant for the carbamates and thiocarbamates. However, bond cleavage with rearrangements analogous to those reported by **Benson** and **Damico** (1968) were observed. For example, S-phenylmethylthiocarbamate gives an intense rearrangment peak at m/e 110 ($C_6H_6S^{+}$), formed via a four- or six-centered transition pathway from the molecular ion. On the other hand, alpha-cleavage predominated for *S*-phenyl *N,N*-dimethylthiocarbamate with the formation of the *N,N*-dimethylisocyanate ion (m/e 72). Hydrogen rearrangement to the aniline group, a reaction similar to that observed in the aryl-*N*-methyl carbamates, is a dominant feature of the mass spectra of *N*-aryl-*N′*-methyl ureas. A comparison spectrum of *p*-nitroaniline provided support that this rearrangement involves a four-membered transition state to form an aniline fragment ion rather than a six-membered transition state to form the alternative fragment:



An interesting rearrangement involving cyclization following cleavage of a chlorine atom was observed for the urea, *N*-(2-chlorophenyl)-*N*-methylurea. Subsequent decomposition of this fragment yielded the HNCO (m/e 43) base peak ion as determined by accurate mass measurements:



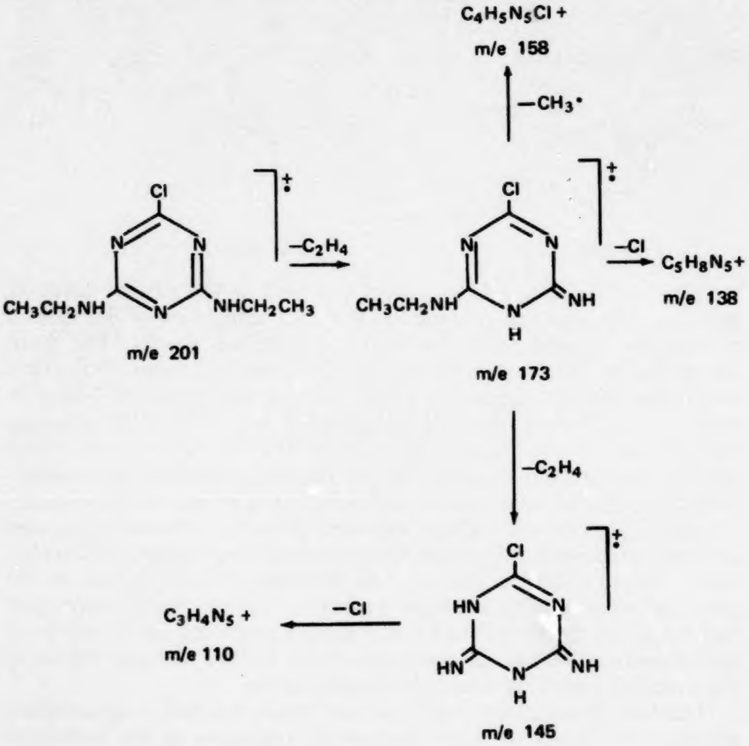
Mass spectral data for several herbicidal carbamates and for monomeric and polymeric fungicidal dithiocarbamates were also obtained. The pesticide Eptam dissociates on electron impact to form the m/e 128, di-*n*-propylisocyanate ion which in turn can undergo simple bond cleavage to form the *n*-propyl ion (m/e 43) base peak or rearrangement to form the species C_3H_7—NH=C=O+ (m/e 86). Alternatively, the molecular ion can undergo a six-membered transition state hydrogen transfer to form a fragment of m/e 161 with the composition: $(C_3H_7)_2$—N—C(OH)=S+.

The metal-containing fungicidal dithiocarbamates, ziram and ferbam, dissociate upon electron bombardment in a manner characteristic of the *N,N*-dimethyl carbamates. Thus, characteristic fragments are observed corresponding to species such as $(CH_3)_2N{=}C{=}S^{+\cdot}$, m/e 88, and $(CH_3)_2N^{+\cdot}$, m/e 44. An additional reaction involving loss of a single $(CH_3)_2NCS_2$ fragment from the parent ion was also detected. Because of the polymeric nature of the fungicides zineb and maneb as well as their thermal instability at elevated temperature, the fine detail of their mass spectra was not analyzed. No molecular ion peaks were observed for these two compounds. Predominant fragment ions observed were CS_2 (m/e 76), ethylene bisdiisothiocyanate (m/e 144), and methylene isothiocyanate (m/e 72).

d) Miscellaneous pesticides

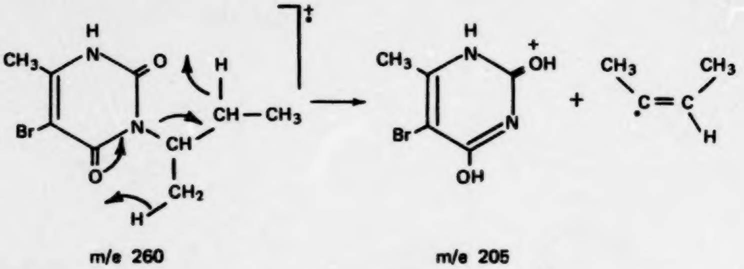
The mass spectra of several other classes of organic compounds used as pesticides along with their fragmentation mechanisms have been reported in the literature although the extent of study within any given group has been less extensive than that for the classes of pesticides discussed above. JORG *et al.* (1966), in their examination of the mass spectra of several classes of pesticides, included information pertinent to six triazine compounds used as herbicides. Under electron impact-induced ionization, relatively intense molecular ion peaks are observed and the major dissociative processes directly involve the substituents on the triazine ring. For example, the molecule propazine undergoes decomposition with loss of propylene to yield a fragment of m/e 187. Subsequent dissociative processes involve loss of a chlorine atom (m/e 152), loss of methyl radical (m/e 172), and a second hydrogen rearrangement with loss of propylene (m/e 145). The base peak (m/e 214) arises from cleavage of the methyl radical from the molecular ion. A relatively intense doubly charged fragment at m/e 99.5 due to loss of two methyl radicals was also observed. An apparently significant cleavage involving formation of the species NH—CH—$(CH_3)_2^+$ resulted in an intense fragment ion at m/e 58.

Additional fragments apparently characteristic of the diaminotriazine skeleton included an imidazole structured ion (m/e 68) and a 1,2,4-triazole ion (m/e 69, $C_2H_3N_3^+$). A partial dissociation scheme for simazine is illustrative of this compound group:


C4H5N5Cl +
m/e 158
−CH3•
Cl
N
N
CH3CH2NH
N
NHCH2CH3
m/e 201
−C2H4
Cl
N
N
CH3CH2NH
N
H
NH
m/e 173
−Cl
C5H8N5+
m/e 138
−C2H4
Cl
HN
N
HN
N
H
NH
m/e 145
−Cl
C3H4N5 +
m/e 110

Additional discussion and mass spectral data were presented by the authors for the compounds atrazine, ametryne, Prometone, and Prometryne.

REISER (1969) investigated the mechanisms of the major fragmentations observed for several selected uracil compounds including the herbicides bromacil and terbacil. The most significant fragment ions for both herbicides are found at M-55 and M-56 arising from cleavage of the alkyl group bonded directly to ring nitrogen 3 accompanied by a rearrangement of one or two hydrogen atoms from the alkyl group to the uracil ring. The single hydrogen rearrangement can be represented as a conventional "McLafferty rearrangement." The double rearrangement, interestingly, involved the migration of the hydrogen atoms to the carbonyl oxygen through concurrently operating five- and/or six-membered cyclic transition states. The rearrangement for bromacil can be illustrated by the following scheme:



That the stability of the radical fragment is the driving force in this double rearrangement was decided by examination of the spectra of 3-methyl, 3-ethyl, and 3-*n*-propyl substituted uracils. The peak due to double rearrangement was not observed for 3,6-dimethyl uracil where the neutral fragment is a CH_3 radical, was weak for 3-ethyl-6-methyl uracil where the neutral fragment is a $CH_2{=}CH\cdot$ ethylene radical, and was the base peak in 6-methyl-3-*n*-propyl uracil where the expelled neutral fragment is a resonance-stabilized allyl radical. The molecular ion of 1- and 3-alkyl substituted uracils such as bromacil and terbacil do not undergo the retro Diels-Alder reaction expected of these compounds because of the preferential rearrangement mechanisms discussed above. Instead, the fragment formed by loss of the alkyl substituent with a single hydrogen rearrangement undergoes this reaction (to lose HNCO), and form a fragment ion of relatively low abundance. Other processes involving further decompositions of the uracil ring result in ions of lower m/e values.

Further observations made on the mass spectral fragmentation patterns of a series of uracil compounds analogous to the herbicides bromacil and terbacil showed that characteristic decomposition reactions would be useful in differentiating among isomeric structures, such as bromacil and 5-bromo-1-*sec*-butyl-6-methyluracil, as well as 5-bromo-3-*sec*-butyl-6-methyl-2-thiouracil and 3-*sec*-butyl-6-methyl-4-thiouracil, among others.

Other mass spectral studies of pesticides have been reported including discussions of the fragmentation behavior of nicotine (McLAFFERTY 1956) and other nicotinoid compounds (BUDZIKIEWICZ *et al.* 1964). Additionally, REED and WILSON (1963) have provided mass spectral data relating to the fragmentation mechanisms of some insecticidal rotenoids.

III. Mass spectrometric technique: Isolation of individual residues

a) Standard isolation and separation procedures

The analysis of individual pesticide residues and metabolic products by mass spectrometry is generally performed by the use of one of a wide variety of sample introduction techniques, such as direct probe inlets, molecular leak systems, and standard batch inlets. The choice of technique is dictated by a consideration of a number of sample characteristics including size, volatility, thermal and chemical stability, purity, source, information desired, etc. An extensive discussion of the advantages and disadvantages of the available mass spectrometric inlet systems other than the direct gas chromatographic inlet (*vide infra*) will not be included in this review. Detailed discussions of this topic, however, are available in literature reviews and texts (McFADDEN 1966, BEYNON 1960). Notwithstanding the advantages of direct gas chromatographic-mass spectrometric analysis of mixtures of organic compounds, in many instances the best approach to the analysis of individual residues involves prior separation of the component of interest followed by the use of a batch introduction system. Direct probe techniques, for example, are particularly suitable for analysis of certain types of compounds because the close proximity of the sample to the ion source permits the analysis of many thermally sensitive compounds with a minimum of decomposition. Most standard chromatographic procedures are applicable to the isolation and separation of individual pesticide residues for subsequent mass spectral analysis. No further consideration will be given here to these individual techniques such as column chromatography and paper and thin-layer chromatography. Literature reviews of these topics are available, however (MORLEY 1966, ABBOTT and THOMSON 1965).

b) Collection devices

The following discussion will be limited to a consideration of some available microcollection devices and methods applicable to the isolation of individual pesticide residues for subsequent mass spectral analysis. The most commonly used techniques involve separating the components of mixtures by means of gas chromatographic methods and trapping the desired peak by means of a suitable collection device designed to retain the sample until mass analysis can be performed. In addition to gas chromatography, collection devices utilizing thin-layer chromatographic separations have also been reported.

AMY *et al.* (1965) have described a fraction collection method involving condensation of gas chromatographic effluent components onto column packing material contained in a glass melting point capillary tube. If the same packing material contained in the column is used, the capillary can be considered to be an extension of the gas chromatographic column, from which the appropriate portion of the sample is removed when it contains the desired component. The capillary collector, removed and flame sealed, provides a convenient storage container until analyses can be performed. With this system, samples as little as 0.1 μg. of pregnanediol diacetate were satisfactorily examined by high resolution mass spectrometry. With photoplate recording, samples of 0.01 to 10 μg. at 150°C. gave a high resolution spectrum useful for qualitative analysis.

Halogenated pesticide residues including DDT, DDE, and dieldrin were successfully identified by mass spectrometry when a similar collection device was used to trap these residues from extracts of alfalfa and wheat. Residues as low as 0.1 μg. were successfully examined (MUMMA and KANTNER 1966). BIERL *et al.* (1968) recently announced a gas chromatographic peak collection device designed to provide efficient recoveries of samples as low as 10 ng., of variable polarity and volatility, and at carrier gas flow rates of 30 to 100 ml./min. Fractions were collected in a short column (trap) of cold packing material and flushed into an analytical instrument such as the mass spectrometer by rapidly heating the packing and passing through it a syringe full of carrier gas. An extensive recovery study was performed employing different trap packings, trap lengths, chromatograph flow rates, and trap heater temperatures. Recoveries ranged from 32 to 106 percent for various classes of compounds including hydrocarbons, alcohols, ethers, and esters. These extensive recovery studies emphasize the lack of sufficient performance data for many of the available gas chromatographic peak trapping devices, which in many instances leaves their range of applicability in some doubt. Factors which may influence recovery include such variables as flow rate while trapping, class of compound, volatility, and size of the sample. In the absence of these data the analyst may frequently be confronted with the need for evaluating a given device for his own application.

Finding that gas chromatographic column packing materials were poor adsorbents for some volatile compounds, DAMICO *et al.* (1967)

elected to trap column effluents in a glass capillary tube containing activated coconut charcoal. Propionaldehyde, 2,3-dimethylbutane, and dimethyl sulfide in quantities less than ten μg. were efficiently trapped by this method. Although detectable quantities of CO_2 were also adsorbed on the charcoal, a stated advantage of the technique is that highly volatile compounds can be introduced into the mass spectrometer without rapid volatilization because of the slow rate of desorption of these materials from charcoal.

Several techniques are available for the mass spectral examination of samples isolated by thin-layer chromatography techniques. **Rix** *et al.* (1969) described a method whereby the material under study is separated as completely as possible by thin-layer chromatography after which the appropriate spots are removed from the plate and transferred to an elution column consisting of a shortened Pasteur pipet drawn out at its lower end to facilitate sample handling. The adsorbent is supported on a small plug of glass wool and eluted with methanol to remove the adsorbed material. After elution of the compound is complete, the lower portion of the column is broken off and the enclosed eluate is evaporated on the top of the solid introduction probe of the mass spectrometer. The technique reportedly gives satisfactory mass spectra for any substance amenable to mass spectrometric analysis and to straightforward thin-layer chromatographic separation.

Rapid indentification of compounds separated by thin-layer chromatography can be provided by eliminating the solvent elution step. **Deverse** *et al.* (1967) described the application of this approach to the mass spectral analysis of benzil and biphenyl. The unknown sample was separated into its individual components on TLC plates. Each spot to be analyzed, including the adsorbent, was transferred to a capillary sampler which was then placed in a controlled temperature inlet system. Submicrogram quantities of sample may be analyzed by using the controlled temperature inlet to control the sample volatilization rate.

Odland *et al.* (1969) have described a trapping technique for the isolation of gas chromatographic samples for subsequent rechromatography or mass spectral analysis. The fractions are collected in a stainless steel U-trap, $\frac{1}{16}$ in. o.d. by 12 in. long, connected to an outlet splitter of a capillary column. Sample peaks condensed by means of Dry Ice can be capped in the trap and stored for later analysis or subjected to rechromatography by use of a specially prepared trap flush inlet adapter. The trapped components are swept into the gas chromatographic column by means of carrier gas at the same time the trap is heated in a Dewar flask of boiling water. Alternatively the trap can be connected to an appropriate $\frac{1}{4}$-in. direct inlet valve on the mass spectrometer. By this technique trapping efficiencies for samples as small as 0.4 μg., having boiling points ranging from 64 to 240°C., were found to be 60 percent regardless of the trap configurations.

SCANLAN *et al.* (1968) reported a variation of the latter technique for overcoming difficulties encountered in the combined capillary column gas chromatographic-mass spectrometric analysis of extracts in which relatively few of the components make up the majority of the total sample. Other components of interest, when present in small amounts, may not be detected by the mass spectrometer under these conditions. This problem was overcome by separating a relatively large sample of the extract on a packed column, collecting the compounds present in small concentrations as they eluted from the packed column and transferring these compounds to a 0.01-in. i.d. capillary column for combined gas chromatographic-mass spectrometric analysis. The packed column fractions were collected in six-in. lengths of 0.03-in. i.d. stainless steel capillary tubing, placed in line with the capillary column, and heated by means of a forced air heat gun to flush the samples onto the column. Excellent mass spectra were obtained for one-μg. quantities of such compounds as 2-heptanone, 2-undecanone, and diacetyl with recoveries ranging from 39 to 91 percent.

WILLIS (1968) described a similar technique employing trapping loops for the collection of samples subjected to a preliminary separation or chemical treatment such as microcatalytic hydrogenation. An improved technique utilizing noncatalytic glass collection traps which may be directly coupled to the mass spectrometric inlet and which exhibits high analytical sensitivity has recently been reported (WOOLEY 1969). A length of thin-walled stainless steel tubing was attached to the exit port of a gas chromatograph to direct the effluent into a glass collection trap cooled in liquid nitrogen. Details of the collection unit provided for venting of the carrier gas, heating of the collection line, and easy removal and capping of the borosilicate collecting tubes. The described technique, when used in conjunction with a mass spectrometer modified to permit rapid analysis of submicrogram samples, provided a maximum working sensitivity of 0.1 μg. and collection efficiencies of 95 percent for hexane and methyl acetate.

Several additional trapping systems for collecting gas chromatographic fractions for mass spectrometer analysis have been described (BURSON and KENNER 1969, CARTWRIGHT and HEYWOOD 1966). A relatively simple and efficient fraction collection system employing Pasteur disposable pipettes to trap specific components of gas chromatographic column effluent has been used successfully in this laboratory in pesticide residue analysis requiring spectroscopic determinations. This technique, in conjunction with column effluent splitters or valving systems, represents a modification of the KABOT (1967) method.

It is apparent from this brief discussion of available trapping techniques that a great deal of interest has been directed toward developing rapid, efficient, and sensitive methods for the isolation and collection of individual organic compounds for mass spectral analysis. The value of the separation and trapping approach to analysis of individual compounds has been demonstrated by its successful utilization in many and varied applications. However, it should be considered that no single trapping technique is suitable for all applications for reasons ranging from specific instrument configurations and requirements to special sample considerations. Thus, in most cases, the analyst will be faced with the problem of evaluating a given technique for his specific research needs.

IV. Applications of mass spectrometry to pesticide residue analysis

Many examples of the successful application of mass spectral analysis to the structure elucidation and characterization of pesticide metabolic and photodecomposition products may be found in the literature. Furthermore, because of the relatively high sensitivity and immense compound structure information content, coupled with the increasing availability of instrumentation and improved instrumental technology, use of mass spectrometry for structural characterization of unknown materials in the field of pesticide research can be expected to increase in the future. As the following discussion will show, when sufficient sample is available mass spectral data should be considered as an extremely valuable complement to information obtained on the same sample by other instrumental and analytical techniques such as nuclear magnetic resonance and infrared and ultraviolet spectrophotometry, as well as the gas and thin-layer chromatographic behavior of the compound, its chemical properties, and other data; it should not be relied on exclusively for structural characterization save in those instances where confirmation alone is sought or where sample size and other considerations require such a single instrumental approach. Additionally, supporting spectroscopic data should be obtained with an authentic sample of the material in question wherever possible.

a) Animal metabolism of pesticides

Employing standard residue isolation and separation procedures such as extraction, liquid-liquid partitioning, column and thin-layer chromatography, Richardson *et al.* (1968 a and b) isolated two metabolites of dieldrin from the urine and feces of rats which had been dosed with 100 p.p.m. of dieldrin for six months. The urinary metabolite examined by both infrared and mass spectrometry was assigned the structure 1,9,10,10,11-*exo*-pentachloro-4,5-epoxy-pentacyclo-$(7,3,0,0^{2,6},0^{3,8},0^{7,11})$-dodecan-12-one. The mass spectrum indicated a molecular weight of 358 amu corresponding to a species containing five chlorine atoms on the basis of the isotopic distribution of the molecular ion peaks, and consistent with the empirical formula, $C_{12}H_7O_2Cl_5$. Further examination of the mass spectrum revealed major diagnostic fragment peaks at m/e 305 and m/e 295. The fragment of m/e 305 (M-53) contained four chlorine atoms and corresponded to the elimination of Cl and H_2O from the molecular ion. This observation was considered to be evidence for the presence of a 1,2-epoxy group on the basis of a similar fragmentation pathway noted for dieldrin. The fragment of m/e 295 also contained four chlorine atoms and represented a loss of 63 amu from the parent ion, which could arise by cleavage of a chlorine atom and a neutral CO molecule. This observation suggested the presence of a carbonyl group within the metabolite. Infrared evidence corroborated the presence of the carbonyl and 1,2-epoxide functional groups in the molecule as well as the absence of unsaturation. On the basis of these data, the metabolite was assigned the aforementioned structure. The unique position of the carbonyl function (C-12) was deduced by eliminating other possible structures.

Simultaneously, but independently, DAMICO *et al.* (1968) presented spectroscopic evidence including mass spectral data for an identical compound isolated from the urine of rats maintained on diets containing dieldrin. Additional evidence elaborated by these authors in support of the assigned structure was the identity of the base peak (m/e 81) in the mass spectrum which had been shown to be due to a protonated cyclopentadienone ion in a study of the fragmentation behavior of epoxides of the dimethanonaphthalene type (e.g., dieldrin). A similar, presumably isomeric metabolite of aldrin isolated by these investigators from a concurrent aldrin metabolism study was also reported. The minor spectroscopic differences noted for the two metabolites were attributed to alternative *exo-* or *endo-*orientations of the epoxide ring. RICHARDSON and co-workers (1968 a) further isolated and partially characterized a second metabolite of dieldrin found to be excreted in feces of rats. Supportive infrared and nuclear magnetic resonance evidence suggested the presence of a 1,2-epoxide group, tertiary alcohol function, and CCl=CCl unsaturation. The mass spectrum of this metabolite consisted of a parent peak at m/e 394 containing the isotopic distribution characteristic of six chlorine atoms. Accurate mass measurement of the M-Cl fragment group (m/e 359) led to a probable empirical formula for the compound of $C_{12}H_8Cl_6O_2$. On the basis of this information, it was suggested that the fecal metabolite was a monohydroxy derivative of dieldrin substituted in position 4a or 5.

BARON *et al.* (1969), in the course of a study involving the occurrence of metabolites of carbaryl in the milk and urine of the dairy cow, presented confirmatory spectroscopic data which identified a major metabolite as the methyl carbamate of 5,6-dihydro-5,6-dihydroxy-1-naphthol. In this study data from low and high resolution mass analysis, field ionization studies, low voltage mass spectra and the mass spectra of model compounds were utilized in addition to infrared and nuclear magnetic resonance studies to establish the structure of the metabolite. High resolution mass analysis confirmed an elemental composition of $C_{12}H_{13}NO_4$ (m/e 235·08445) for the metabolite. The fragmentation pattern of the isolated compound was similar to that observed for other known aryl methyl carbamates as well as the model compounds 5-hydroxycarbaryl and 1,2-dihydro-1,2-dihydroxynaphthalene. The parent ion species undergoes hydrogen rearrangement to yield the corresponding phenol (m/e 178) with loss of methylisocyanate. Successive fragmentation of the peak at m/e 178 involved loss of water to form an ion at m/e 160 corresponding to the base peak and an ion at m/e 132 due to loss of CO. Metastable transitions were observed for these processes. A fragment ion at m/e 217 was reconciled with loss of water from the molecular ion as a result of both electron impact and thermal decomposition.

Further applications of mass spectrometry for the characterization of pesticidal metabolites have been reported particularly in studies involving the metabolic fate of herbicides in the rat and dog. In research aimed at determining the *in vivo* biochemical transformation of siduron in the dog, BELASCO and REISER (1969) characterized three metabolites on the basis of thin-layer chromatography, nuclear magnetic resonance, infrared, and mass spectral techniques.

Table I. *Principal mass spectral peaks and their identities observed for Siduron and three metabolites isolated from dog urine*

Compound	m/e and identity
Siduron	232 (M+), 93 ($C_6H_5NH_2$), 119 (C_6H_5NCO), 135 ($C_6H_5NHCONH$)
1-(4-Hydroxy-2-methylcyclohexyl)-3-(*p*-hydroxyphenyl) urea, A	264 (M+), 109 ($HOC_6H_4NH_2$), 135 (HOC_6H_4NCO), 155 ($[HO][CH_3]C_6H_9NCO$), 129 ($[HO][CH_3]C_6H_9NH_2$)
1-(4-Hydroxy-2-methylcyclohexyl)-3-phenylurea, B	248 (M+), 93 ($C_6H_5NH_2$), 119 (C_6H_5NCO), 135 ($C_6H_5NHCONH$)
1-(*p*-Hydroxyphenyl)-3-(2-methylcyclohexyl) urea, C	248 (M+), 109 (*p*-$HOC_6H_4NH_2$), 135 (*p*-HOC_6H_4NCO)

The mass spectral data, briefly summarized in Table I, exhibited fragmentation processes typical of phenylurea structures. Molecular weight differences of 16 amu (for metabolites B and C) and 32 amu (for metabolite A) suggested that the metabolites were hydroxylated derivatives of the parent herbicide. The base peak of siduron and metabolite B, m/e 93, corresponded to an aniline ion. Both compounds also exhibited major fragments at m/e 119 and m/e 135. Because metabolite B was a hydroxylated derivative of siduron, the hydroxyl function was thus deduced to be located on the cyclohexyl ring portion of the molecule.

NMR evidence was required to locate the position of the hydroxyl function and the structure of metabolite B was determined to be 1-(4-hydroxy-2-methylcyclohexyl)-3-phenylurea. The structure of metabolite C was determined to be 1-(*p*-hydroxyphenyl)-3-(2-methylcyclohexyl) urea on the basis of comparison of the mass spectrum of the unknown with an authentic sample. Although an isomer of 1-(*o*-hydroxyphenyl)-3-(2-methylcyclohexyl) urea had an essentially identical mass spectrum to that of metabolite C, the vaporization temperature (115° to 120°C.) required in the direct probe analysis of the compound was in contrast to that observed for both the sample and the *p*-hydroxy analog (85°C.). Metabolite A, a double hydroxylated derivative of siduron, appeared to be a metabolic daughter of metabolites B and C and thus was assumed to be 1-(4-hydroxy-2-methylcyclohexyl-3-(*p*-hydroxyphenyl) urea. Supportive infrared, thin-layer, and NMR data were also obtained for this compound.

Several reports have appeared in the literature relating to the application of mass spectrometry in characterization of metabolites of uracil-type herbicides in animals. GARDINER *et al.* (1969) studied the metabolic fate of bromacil in rats fed 1,250 p.p.m. in their diets, and partially on the basis of mass spectrometry, structurally identified a total of six metabolites of bromacil in the rat urine. Characteristic mass spectral fragmentation patterns of a series of substituted uracils (REISER 1969) were used extensively in establishing structural identification. A major metabolite was identified as 5-bromo-3-*sec*-butyl-6-hydroxymethyl uracil on the basis of comparison of the spectral properties of the unknown material with an authentic sample as well as comparison with the mass spectral data of the parent, bromacil. This metabolite exhibited a molecular ion of m/e 276 indicative of a hydroxylated bromacil species. It was possible to establish the fact that the bromine atom and *sec*-butyl groups were intact in the metabolite on the basis of similarities in the fragmentation scheme observed for bromacil and the metabolite, thus suggesting that the additional oxygen atom was bonded to the 6-methyl substituent.

A second metabolite was shown to be 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil on the basis of the mass spectral fragmentation pattern and other pertinent spectroscopic data. The molecular ion doublet of m/e 276 and m/e 278 indicated that an oxygen atom had been incorporated in the metabolite and that the bromine atom remained intact. The absence of a M-55 fragment characteristic of loss of a *sec*-butyl group in the parent bromacil and the observation of M-71 and M-72 fragments indicated that the oxygen atom was bonded to one of the carbon atoms of the *sec*-butyl group. The observation of a relatively abundant fragment ion at M-44 (CH_3CHO) further suggested that the additional oxygen atom was attached to the secondary carbon atom in the *sec*-butyl group.

5-Bromo-3-(2-hydroxy-1-methylpropyl)-6-hydroxymethyluracil was also identified in the rat urine extracts and showed mass spectral characteristics consistent with the assigned structure, including a molecular ion of m/e 294 and fragment peaks of M-44 (loss of CH_3CHO), M-71 (loss of C_4H_7O, hydroxylated *sec*-butyl group) and M-115 (retro Diels-Alder cleavage). The compounds 3-*sec*-butyl-6-hydroxymethyluracil (M+, m/e 198), 5-bromo-3-(3-hydroxy-1-methylpropyl)-6-methyluracil (M+, m/e 276), and 3-*sec*-butyl-6-methyluracil (M+, m/e 182) were shown to be minor metabolites of bromacil with experimental evidence relying to a significant degree on the mass spectral fragmentation behavior of these compounds.

Rhodes *et al.* (1969) have reported analogous detailed studies of the metabolic fate of terbacil in dogs in which mass spectrometry played an important role in establishing the structures of a total of five metabolites of the herbicide isolated from dog urine. The individual residues were extracted and purified by means of thin-layer chromatographic separation. Identities as well as significant mass spectral data are contained in Table II. The metabolic pathway of terbacil proceeds in a fashion similar to that of bromacil with the major product consisting of the hydroxylated derivative, 3-*t*-butyl-5-chloro-6-hydroxymethyluracil. Other significant metabolites contained the substituted bicyclic oxazolopyrimidine structure formed from initial hydroxylation of a primary carbon atom on the *t*-butyl group coupled with elimination of a molecule of water. A significant dechlorinated metabolite (E) was also isolated. In this study as well as the preced-

Table II. *Selected mass spectral data for terbacil and four metabolic conversion products isolated from dog urine*

Compound	m/e and identity
Terbacil	216 (M+), 161 ($M-C_4H_7$), 117 ($M-C_4H_7NCO$)
3-*t*-Butyl-5-chloro-6-hydroxy methyluracil, A	232 (M+), 177 ($M-C_4H_7$), 133 ($M-C_4H_7NCO$), 149
6-Chloro-2,3-dihydro-7-(hydroxymethyl)-3,3-dimethyl-5-H-oxazolo-(3,2-a) pyrimidin-5-one, B	230 (M+), 201 (M-CO), 175 ($M-C_4H_7$), 160, 147 ($201-C_4H_6$)
6-Chloro-2,3-dihydro-3,3,7-trimethyl-5-H-oxazolo (3,2-a) pyrimidin-5-one, C	214 (M+), 199 ($M-CH_3$), 144 ($199-C_4H_7$)
3-*t*-Butyl-5-hydroxymethyluracil, D	198 (M+), 173 ($M-CH_3$), 143 ($M-C_4H_7$), 125, 116
3-*t*-Butyl-6-formyluracil, E	196 (M+), 141 ($M-C_4H_7$), 110

ing one involving the herbicide bromacil, the known electron impact fragmentation behavior of uracil compounds contributed substantially to the analysis of the mass spectra of the unknown metabolites.

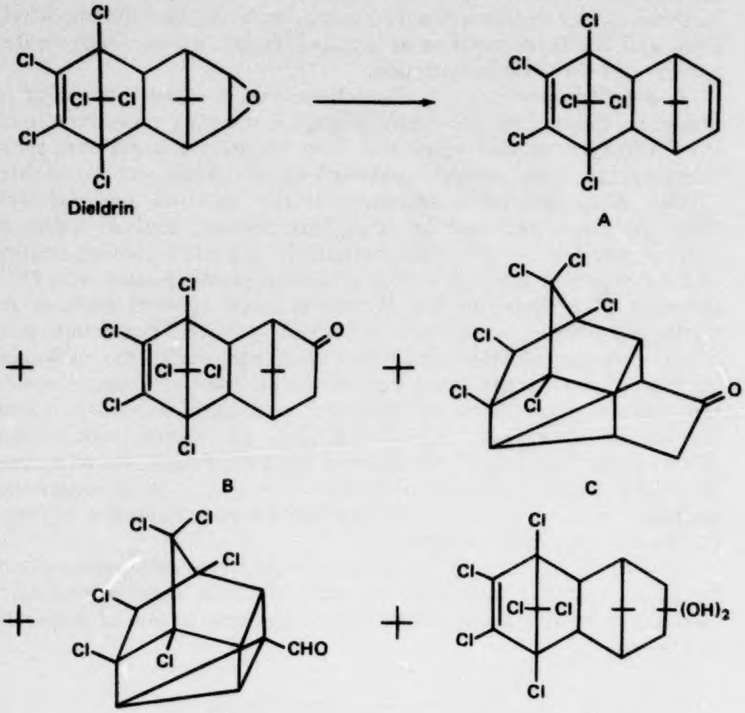
Additional studies in which mass spectrometry has aided in the analysis of individual pesticidal residues have involved carbaryl metabolism in poultry (PAULSON *et al.* 1970) and dieldrin-^{14}C metabolism in sheep (FEIL *et al.* 1970). BALDWIN and ROBINSON (1969) employed mass spectrometric methods in the characterization of metabolites of photodieldrin in rats.

A recent note (LAWRENCE *et al.* 1970) describes the identification of a chlordane metabolite found in milk and cheese and characterized by mass spectrometry, infrared spectrophotometry, and chemical synthesis. Similarities of the fragmentation pattern of this compound with other members of the methanoindene class of insecticides coupled with a molecular ion of m/e 420 and a base peak of m/e 115 (compare the base peak of m/e 81 observed for heptachlor epoxide) containing one chlorine atom and arising from retro-Diels-Alder reaction of the M-Cl ion helped to establish the structure of the metabolite as 1-*exo*-2-*endo*-4,5,6,7,8,8-octachloro-2,3-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene.

b) Plant and soil induced chemical alteration of pesticides

The importance of mass spectrometry in the characterization of terminal residues of pesticides formed by plant metabolism reactions and the chemical influence of soil microorganisms is well established.

WHEELER *et al.* (1967) and MUMMA *et al.* (1966) applied mass spectrometric analysis to the identification of residues of dieldrin in a study aimed at determining absolute efficiencies of extraction procedures for internal accumulations of this insecticide in plants via root uptake. MATSUMURA *et al.* (1968) studied the effects of both soil and soil bacteria on the chemical decomposition of dieldrin and reported mass spectral evidence in support of structures for five conversion residues. Chemical, chromatographic, and infrared data in conjunction with mass spectrometric fragmentation data established the formation of aldrin, A, a ketodieldrin, B (M+, m/e 378), a photoketodieldrin, C (M+, m/e 378), and a dieldrin aldehyde, D (M+, m/e 362). Observation of prominent peaks at M-28 (m/e 350) and M-63 (m/e 315), as well as comparison with the mass spectrum of the known ketoendrin (ROSEN *et al.* 1966), established the structure for the ketodieldrin. The mass spectrum of ketodieldrin was essentially identical to that of the photoketodieldrin. Further, the observation of a photochemically induced conversion of the ketodieldrin into the photoketodieldrin and analogy with the known conversion of dieldrin and aldrin into "bridged isomers" under the influence of ultraviolet irradiation suggested that the photoketodieldrin also contained the bridged structure.



Dieldrin

A

B

C

D

E

The mass spectrum of the dieldrin aldehyde, D, exhibited prominent fragment peak groups at M-63 and M-77, suggesting that a loss of one chlorine was followed by the expulsion of both CO and CH_2CO. Additional chemical and infrared data led to a tentative proposal for the structure as indicated above. Information for the dihydroxydieldrin, E, included a prominent ion group at m/e 377 with six chlorine atoms, and mass spectral clusters at m/e 361 and m/e 325 with five chlorine atoms, implying a dihydroxydieldrin structure similar but not identical to dihydrodihydroxy aldrin.

The metabolism of diuron applied to corn seedlings was extensively studied by ONLEY *et al.* (1968). Gas chromatography, infrared spectrophotometry, radioactivity measurements, and mass spectrometry were utilized in establishing the identities of individual metabolites. Gas chromatographic trapping procedures were employed for isolation and collection of residues subsequently characterized by infrared and mass spectrometry. Comparison with the mass spectra of standard materials permitted identification of 3,4-dichloronitrobenzene, 3-(3,4-dichlorophenyl)-1-methylurea, 3-(3,4-dichlorophenyl) urea, and 3,4-dichloroaniline as metabolites in corn seedlings treated with diuron via a nutrient solution.

A detailed spectrometric characterization of a butyl homolog of malathion isolated by gas chromatographic trapping procedures both from field-sprayed kale crops and from commercial malathion spray solutions has been recently reported by GARDNER and co-workers (1969). Mass spectral comparisons of the unknown material with those previously reported for phosphorodithioate methoxy esters as well as inherent sample data tentatively provided characterization of the compound as ethyl butyl mercaptosuccinate, S-ester with *O,O*-dimethyl phosphorodithioate. Prominent mass spectral features included a molecular ion of m/e 358, M-45 and M-73 peaks indicative of the presence of ethoxy and butoxy groups within the molecular structure, an m/e 201 (base) peak arising from cleavage alpha to the thiolate sulfur atom to form the ethyl butyl succinate radical ion, an m/e 125 peak, $(CH_3O)_2PS^+$, and an intense peak of m/e 155 resulting from loss of ethyl alcohol from the base peak and further providing evidence for the presence of a butoxy group within the molecule. Infrared and NMR data provided further definitive evidence for the structure of this compound.

Studies of the metabolism of halogenated aromatic hydrocarbons by microorganisms have been reported in which mass spectrometry (GIBSON *et al.* 1968) was utilized in the characterization of metabolic products.

c) *Photodecomposition of pesticides*

Because pesticides in the environment are exposed to sunlight for extended periods, it is important to establish the identity and toxicity of compounds formed by photochemical degradation. Increasing interest in the study of these photochemical reactions has emphasized the utility of mass spectrometry for characterizations of the unknown transformation products. ROBINSON and associates (1966) proposed a structure for photodieldrin, a photoisomerization product of dieldrin, based partially on observations of the electron impact-induced fragmentation modes for the conversion product when compared with the mass spectra of the standard materials dieldrin and endrin.

Simultaneously, ROSEN *et al.* (1966) published data supporting an identical structure for the photoisomerization product of dieldrin. Significant mass spectral peaks observed included an m/e 378 molecular ion, isomeric with dieldrin, as well as a base peak of m/e 81 due to the $C_5H_5O^+$ fragment formed by a retro Diels-Alder type decomposition reaction. The more significant proportion of this fragment species produced in the mass spectrum of photodieldrin when compared to that of dieldrin suggested to these authors that bond formation had occurred at the methylene carbon atom to form a hexacyclic structure as indicated in Figure 1. The ultraviolet irradiation of endrin also studied by these researchers resulted in the formation of the pentacyclic ketoendrin and endrin aldehyde species also indicated in the figure.

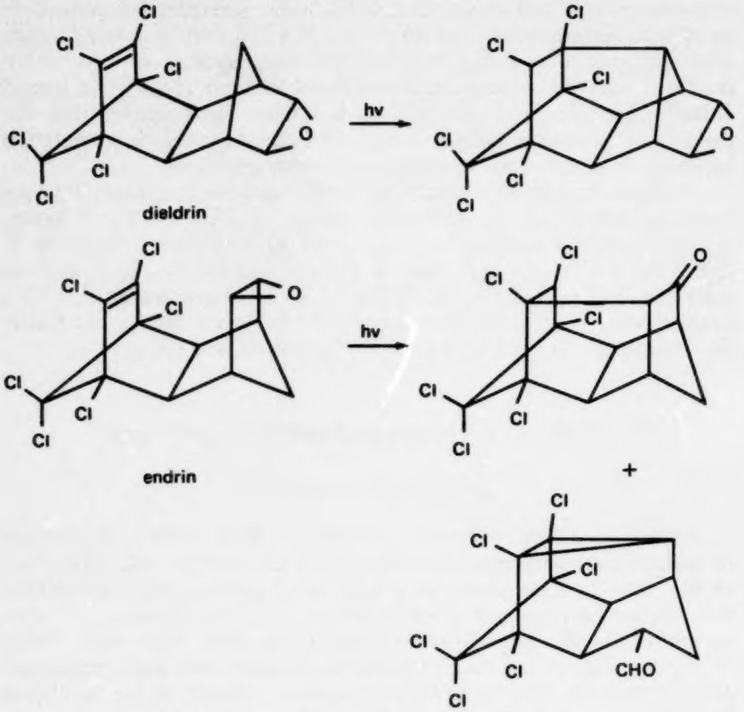


Fig. 1. Photoisomerization of dieldrin and endrin.

The photochemical transformation accompanying the ultraviolet irradiation of solutions of Patoran have been examined by Rosen and Strusz (1968). A major product was found to be 3-(p-hydroxyphenyl)-1-methoxy-1-methylurea on the basis of gas and thin-layer chromatographic and infrared and mass spectrometric properties, compared with those obtained for a synthetically prepared sample. Pertinent mass spectral data included a parent ion at m/e 196 and a base peak at m/e 135 corresponding to *p*-hydroxyphenyl isocyanate. Other photochemical products detected included 3-(*p*-bromophenyl)-1-methylurea and *p*-bromophenylurea. Experimental evidence was also presented for the formation of a substituted diphenyl species by dimerization of two molecules of Patoran followed by loss of hydrobromic acid.

The photolysis reaction of 2-methylthio-*s*-triazines resulting in desulfurization has been investigated by PLIMMER *et al.* (1969). Products of the reaction of simetryne and prometryne isolated by thin-layer chromatography and characterized by mass spectrometry proved to be 2,4-bis(ethylamino)-*s*-triazine and 2,4-bis(isopropylamino)-*s*-triazine, respectively. Comparison of the mass spectra of the photoproducts with authentic samples established their identity. A formal analogy between fragmentation in the mass spectrometer and the process of photodecomposition was drawn when the fragmentation behavior of simetryne on electron impact was analyzed.

Additional reported research in which mass spectrometric analyses have assisted in structural characterization of pesticidal photodecomposition products include studies of the photochemical behavior of diphenamid (ROSEN 1967), diquat (SLADE and SMITH 1967), phenylurea herbicides (ROSEN *et al.* 1969), amitrole (PLIMMER *et al.* 1967), l-naphthalene acetic acid (WATKINS and WOODCOCK 1968), and finally the insecticides dieldrin and aldrin (HENDERSON and CROSBY 1967).

V. Combined gas chromatography-mass spectrometry

a) *Coupling techniques*

Several excellent reviews are available which include discussions of instrumental requirements, techniques, advantages, and limitations of the combined gas chromatographic-mass spectrometric method for the solution of problems of confirmation and identification in a wide variety of specific applications (LITTLEWOOD 1968, MCFADDEN 1966). The magnitude of the current literature dealing with both techniques and applications is witness to the immense interest in the combined method. The succeeding discussion will encompass a consideration of direct gas chromatographic-mass spectrometric coupling techniques and a brief description of available separator systems for interfacing a gas chromatograph with a mass spectrometer. For a more inclusive consideration of interfacial systems for gas chromatograph-mass spectrometer coupling, the reader is referred to a recent comprehensive review by REES (1969).

Mass spectrometry was probably first applied to the analysis of gas chromatographic effluents by indirect techniques in which the sample was collected from the carrier gas stream in a cold trap and the resulting sample transferred to the mass spectrometer using standard vacuum methods. Such devices and techniques have been considered earlier in this report. Direct methods, on the other hand, may use one of two approaches determined by the presence or absence of a molecular separator interface. A quasi-direct gas chromatographic inlet system, reported by MILLER (1963), consists of a series of traps connected directly to the mass spectrometer and designed so that a portion of the gas chromatograph effluent can be isolated by cold trapping and then passed through a leak valve into the mass spectrometer. A series of traps may be fashioned so that several peaks can be sequentially frozen out and admitted into the ion source for analysis. In the interim, carrier gas can be pumped away or vented.

The first published report of analyses performed by a directly coupled gas chromatograph-mass spectrometer system appeared approximately ten years ago (GOHLKE 1959). Since that time, many varied approaches to achieving direct (and indirect) mass spectrometric analysis of gas chromatographic effluents have been devised.

A direct coupling of a gas chromatographic column and a mass spectrometer can be achieved by the use of a system recently described by JOLY (1968). Provided sample size permits, gas chromatographic elution may be performed on a packed column, the exit end of which is connected to a splitter line allowing only a small predetermined portion to enter the mass spectrometer through a gold foil leak.

Interrupted elution gas chromatography as a means of sample transferral has been discussed by SCOTT *et al.* (1967). The individual sample components are eluted and trapped, and the carrier gas flow is stopped; the trapped sample is then transferred to an infrared gas cell and/or allowed to leak into a mass spectrometer. When the spectrum has been obtained, the carrier gas flow is again started and the procedure repeated on elution of the next peak.

Direct coupling of small diameter capillary gas chromatographic columns to a mass spectrometer has proved to be a useful approach in a number of analytical applications. Where the problems are those of small sample size, components at low concentration levels, and components of types that require optimum separability, the improved performance of a direct coupled system is required. SCANLAN *et al.* (1968) have described the utility of a capillary column directly coupled to a mass spectrometer. Sample extract fractions eluted from a packed column were trapped in a capillary tube, cooled, and connected to the injection system of a capillary column; the exit end in turn is directly coupled to a mass spectrometer. The utilization of support-coated open tubular (SCOT) columns directly coupled to a mass spectrometer by means of a capillary restrictor has been described recently by MERRITT *et al.* (1969). Comparison data on four closely boiling isomers of hexane at carrier gas flow rates of two ml./min. showed excellent separation on direct coupling of the SCOT column to the mass spectrometer. The column efficiencies were comparable to those obtained with a separator or in a gas chromatograph although the use of the molecular separator (glass frit type) required a somewhat larger sample for the same response.

A directly coupled gas chromatograph-mass spectrometer requires a chromatogram-type readout analogous to the one produced by a conventional detector in gas chromatography. Most commercially available mass spectrometers provide a means for producing such a chromatogram of the gas chromatographic effluent. A separate ion source, successive mass scan integration, or more commonly, a total ion-current monitor are some of the techniques employed for producing the chromatogram. The use of an auxiliary detector, in- or off-line, is another frequently used approach, particularly in flavor applications. BLACK *et al.* (1969) described the use of a flame ionization detector in tandem with a membrane molecular separator to monitor components of sample extracts in aroma research and other biochemical applications.

Thermal conductivity and electron capture detectors may be used as in-line auxiliary detectors despite difficulties arising from reduced pressures of the exit end of the column. A stream splitter and an off-line flame ionization detector is an arrangement which has been commonly used. In both these systems the problems caused by pressure drops across the column complicate the applicability of the approach. The influence of gas chromatographic-mass spectrometric coupling on the performance of a gas chromatographic column has recently been discussed by SELLIER and GUIOCHON (1970).

LEEMANS and MCCLOSKEY (1967) and FLATH *et al.* (1969) reported the use of a valving system to provide flame ionization detector chromatograms of sample mixtures and simultaneously permit venting of both solvent peaks and components present in a large excess. Thus, high capacity open tubular columns can be utilized without saturating the mass spectrometer with components present in high concentration. The utility of a gas chromatographic-mass spectrometer system with an auxiliary electron capture detector or flame ionization detector providing simultaneous quantitation and confirmation of pesticide residues is obvious but has never been reported to date.

A large number of direct coupling techniques involving the use of molecular separators have been described. The reduction of pressure from atmospheric to that compatible with the ion source of the mass spectrometer and the enrichment of column effluent by removal of carrier gas are the main function of the gas chromatograph-mass spectrometer interface. The choice of interface for a given application depends on a number of instrumental and sample considerations, including sample size, sample component chemical and thermal stability, carrier gas flow conditions, column size, mass spectrometer pumping speed, upper pressure limit in ion source, and ion source design. With regard to specific interface characteristics, such considerations as critical temperature range, range of gas flow rates, enrichment and yield factors, peak lag time, and sturdiness are important. Many different devices are available as interfaces: the jet molecular diffusion separator, porous Teflon tube, heated thin silicone membrane, porous metal membranes, glass frit tube, and dual stage modifications and combinations of the preceding types.

The porous-glass separator has been successfully employed by a number of workers in various applications since it was originally described by WATSON and BIEMANN (1965). This interface undoubtedly is the most widely used and has been the most completely studied. GRAYSON and WOLF (1967), for example, have tabulated the operating characteristics of the fritted glass tube molecular separator. Stated advantages of this system include a wide range of carrier gas flow rates and temperature, no peak lag time, and high enrichments (ratio of relative amounts of sample in the carrier gas in the mass spectrometer and in the separator; ideally this ratio is infinite). Disadvantages include fragility, low yield (ratio of amount of sample entering the mass spectrometer to that entering the separator), adsorption and decomposition of polar compounds, and diminished gas chromatographic column efficiency.

LLEWELLYN and LITTLEJOHN (1966) have reported the use of a silicone rubber diaphragm as a molecular separator. The gas chromatograph effluent components pass through the membrane while the carrier gas is vented to the atmosphere or pumped away. Single and dual stage designs of this separator have been described. Since the exit end of the chromatograph is at atmospheric pressure, this separator is most amenable to the tandem installation of an auxiliary detector. Large differential pressures, however, lead to membrane rupture and development of pinhole leaks. Other disadvantages include sample discrimination, peak broadening, and critical temperature and gas flow requirements (BLACK *et al.* 1969). Advantages include extremely high enrichment factors and good yields.

In the jet-molecular diffusion separator reported by RYHAGE (1964), the column effluent passes through a tiny stainless steel orifice which is critically aligned with another orifice. The lighter carrier gas molecules diffuse away from the main stream of effluent, in contrast to the heavier organic sample molecules; enrichment is thus achieved. With the dual stage design, supersonic gas speeds are achieved, minimizing dead volumes, surface areas, and catalytic effects. The dual stage design is available only on a single type of mass spectrometer, whereas single stage designs are more readily available. High carrier gas flow rates may be used, although inflexibility in this regard may be a disadvantage. Enrichments and yields are good under proper operating conditions of gas flow rate and temperature.

A molecular separator system in which the glass frit of the Watson-Biemann type interface has been replaced with a porous thin-walled Teflon capillary tube has been described by LIPSKY *et al.* (1966). The Teflon capillary connected at both ends to stainless steel capillary restrictor lines is enclosed in a vacuum jacket connected to a mechanical pump. As in the porous glass tube separator, preferential diffusion of the lighter carrier gas molecules accounts for the operating principle. Sturdiness and high yield (50 to 90 percent) are the main advantages of the device whereas appreciable peak lag time and distortion, low enrichment, and critical temperature and carrier gas flow are the system's disadvantages.

Other molecular separators have been described which employ the gas diffusion principle through a porous metal membrane such as a porous stainless steel tube (KREUGER and MCCLOSKEY 1969) and porous silver diaphragm (BLUMER 1968). Additionally, a dual stage combination of two previously discussed separators has been constructed and some characteristics of the system established (GRAYSON and WOLF 1970).

Although many of the systems utilizing porous metal membranes have not been thoroughly evaluated, the chief disadvantages appear to be the metallic (hence catalytic) construction, critical carrier gas flow rates, and low yields. With regard to the application of a specific molecular separator in pesticide residue analysis, no performance data obtained directly with this group of compounds are available in the literature. There is an apparent need for detailed systematic studies to establish an optimum gas chromatographic-mass spectrometric coupling system of utility in the analysis of the several organic compound classes of pesticides.

b) Other instrumental considerations

With recent innovations and developments in instrumental design, mass spectrometry combined with gas chromatography can be considered one of the most sensitive spectroscopic tools available to the analyst, in certain cases providing useful data on one μg. or less of material. Although pesticide residue evaluation by the combined technique has been hampered in the past partly because of instrumental sensitivity considerations, the application of the technique can still be considered to be in an embryonic stage. General concordance that the technique provides one of the best approaches to pesticide residue confirmation will no doubt contribute to its rapid future development in this area of application.

The need for confirmation of chemical identity in ultramicroanalysis has led to the development of several techniques for enhancing the sensitivity of mass spectral data. In addition to inherent limitations imposed by the mass spectrometer, the examination of small quantities of materials by the combined method introduces additional factors which can influence the quality of the recorded mass spectral data.

The mass spectra of compounds emerging from a gas chromatograph will be distorted if a change in concentration occurs during the scanning period. To minimize this distortion, the mass spectrum is scanned rapidly at the top of the peak. Rapid scanning, however, can adversely affect the integrity of the mass spectrum, since statistical fluctuation associated with the recording of small ion currents at fast rates can cause serious errors in the recorded magnitudes of these currents. As a consequence the spectrum becomes less accurate and less reproducible as the scan rate is increased or the sample size McFadden and Day (1964) have made quantitative estimates of the is decreased. Littlewood (1968), Merritt *et al.* (1965), and limitations associated with measurements of low ion currents.

Photographic plate detection provides an ideal approach to overcoming these limitations of sensitivity because several mass spectra can be exposed and thus summed on a single plate and subsequently integrated (Watson and Biemann 1965). A serious limitation is the general unavailability of photoplate detection with combined gas chromatograph-mass spectrometer instruments partly due to the requirement of a specific mass spectrometer design for photoplate data acquisition. By using a mass spectrometer focused on a certain mass number as a gas chromatograph detector, picogram and nanogram quantities of material are frequently detectable. Sweeley *et al.* (1966) have described the mass spectral determination of unresolved components in gas chromatographic effluents by simultaneously recording the changing intensities of the selected m/e values during elution of the mixture from the gas chromatograph. Mixtures of steroidal trimethylsilyl ether derivatives were determined in this fashion with as little as 20 ng. of sample.

Refinements of the technique later reported by Hammar *et al.* (1968) permitted the simultaneous recording of ion intensities for up to three separate mass numbers characteristic of a single substance or group of compounds. Termed "mass fragmentography," this technique allowed confirmation of picogram quantities of chlorpromazine and its metabolites in human blood by focusing on the molecular ion and/or other characteristic fragments in the mass spectrum.

Ratio-recording of the mass spectra of gas chromatographic effluents has been developed by Kennett (1967). In this system spectral distortion caused by concentration changes in the ion source can be avoided by recording individual ion currents as ratios of the total ion current, rather than as their absolute values. Ratio recording allows the scanning time to extend over most of the period during which a compound is emerging from the column, thus enhancing instrumental sensitivity. Directly comparable mass spectra may be recorded for any portion of a gas chromatographic peak.

WIDMARK *et al.* (1968) and BERGSTEDT and WIDMARK (1970) interfaced a gas chromatograph-mass spectrometer system with a multichannel analyzer allowing pulse height analysis of up to ten individual mass spectral peaks at a rate of ten sweeps/second. Alternatively, by using a mass marker, the entire mass spectrum could be repetitively scanned. A characteristic isotopic pattern for the $PbCH_2CH_3+$ ion (m/e 235, 236, 237) of tetraethyl lead was obtained from 300 pg. injected into the combined system.

A recent investigation was made of the utility of a small time averaging computer interfaced with a gas chromatograph-mass spectrometer for the enhancement of sensitivity and the isotopic distribution of selected fragments in the mass spectra of organochlorine pesticides (BIROS 1970). Dieldrin has been reliably identified at levels of 50 ng. injected into the chromatograph-mass spectrometer when the parent ion region (m/e 375–385) was repetitively scanned. Examination of the base peak in the spectrum of *p,p'*-DDE allowed confirmation of one ng. quantities after 24 spectral accumulations.

VI. Applications of combined gas chromatography-mass spectrometry to pesticide residue analysis

Mass spectral data may be used to provide definitive and conclusive confirmation of residue identity as well as characterization of residues of unknown structure. Proper and reliable interpretation is required in the latter case and comparative spectra from correctly chosen standards usually will suffice in the former. Examples of both applications of gas chromatography-mass spectrometry in the pesticide residue field are becoming increasingly common as a result of the availability of relatively inexpensive instrumentation, combining good sensitivity, reliability, and ease of operation. In the following discussion, available data will be reviewed pertaining to the application of gas chromatography-mass spectrometry for analysis of pesticide residues as both a characterization tool and a confirmatory technique.

BARTHEL *et al.* (1969) confirmed the identity of pentachlorophenol in samples of human blood, urine, tissue, and clothing by combined gas chromatographic-mass spectrometric analysis of hexane extracts of these substrates. This report constituted a detailed account of the analytical aspects of an epidemic of infant deaths associated with ingestion of this compound. Analyses of the underivatized phenol were performed on a 1.0 m. × 2.5 mm. spiral glass column packed with three percent DEGS and two percent phosphoric acid. Identity was confirmed on the basis of the observation of four major ions at 20 eV in the mass spectrum of the sample peaks when compared to a spectrum of standard pentachlorophenol. The diagnostic peaks consisted of the molecular ion of m/e 264, an ion of m/e 229, due to loss of a chlorine atom, a major fragment resulting from loss of both HCl and CO at m/e 200, and finally, a characteristic fragment of m/e 165 undoubtedly resulting from loss of a chlorine atom from the m/e 200 ion.

Recently BELLMAN and BARRY (1969) have reviewed the utility of combined gas chromatography-mass spectrometry employing a Watson-Biemann design molecular separator for the analysis of solvent mixtures and pesticide residues of the organochlorine type. Comparative sensitivity studies were performed with the total ion current monitor of the mass spectrometer and a flame ionization detector and related to the response characteristics of both chlorinated and non-chlorinated hydrocarbons with both detection systems. It was determined that the total ion current monitor of the mass spectrometer has approximately the same sensitivity for both classes of compounds. The flame ionization detector, however, showed strong bias against chlorinated hydrocarbons. The loss of gas chromatographic column efficiency and resolution with the use of the glass frit molecular separator was observed to amount to approximately eight percent, resulting from the pressure drop across the chromatographic column and dead volume of the separator and ion source. Peak delay time through the molecular separator and detection limits for the instrument configuration described were also reported by these investigators.

During the course of studies involving the development of procedures for the detection and quantitation in human and animal urine of l-naphthol, a major metabolite of carbaryl, BIROS and SULLIVAN (1970) employed combined gas chromatography-mass spectrometry to confirm the derivatized residue, 1-naphthyl chloroacetate. The analytical method involves acid hydrolysis of urine, benzene extraction, derivatization, and cleanup by silica gel column chromatography. Utilizing programmed temperature analyses with a coiled glass column, 4 ft. × 1/8 in. o.d., packed with two percent ST-30 on 60/80 mesh Gas Chrom Q, residues of 1-naphthol as the chloroacetate ester were confirmed in human urine of individuals occupationally exposed to carbaryl. Diagnostic mass spectral peaks for 1-naphthyl chloroacetate include the molecular ion of m/e 220, a base peak probably equivalent to a l-naphthol radical ion found at m/e 144 arising from hydrogen rearrangement and loss of CHClCO, a relatively weak peak at m/e 127 formed either by elimination of $OCOCH_2Cl$ from the molecular ion or loss of water from a relatively intense m/e 145 fragment, and finally, other peaks characteristic of the fragmentation of 1-naphthol at m/e 115 and m/e 116 arising from expulsion of CHO and CO, respectively, from the m/e 144 fragment, and a peak at m/e 89 presumably resulting from a C_7H_5+ ion.

Combined methodology and metabolism studies in animals involving the herbicides 2,4-D and 2,4,5-T provided an opportunity for application of the combined technique to the confirmation of intact residues and known metabolites and the structural characterization of unknown metabolites isolated from rat urine. The analytical technique (SHAFIK and SULLIVAN 1970), briefly summarized, involves acid hydrolysis of phenolic conjugates, extraction of free phenols and acids with benzene, extract concentration and derivatization with diazoethane, and cleanup and separation by silica gel chomatography utilizing benzene-hexane, and benzene-ethyl acetate solvent systems as eluents. Gas chromatographic programmed temperature analyses of the urine extracts with an aluminum column, 7 ft. × ⅛ in. o.d., packed with 20 percent OV-101 on 60/80 mesh Gas Chrom Q, provided the total ion monitor traces shown in Figure 2. Mass spectral data were used to confirm the presence of 2,4-D and 2,4,5-T as the ethyl esters and 2,4,5-trichlorophenol as the ethyl ether in the several analyses performed (BIROS 1970 b). The diagnostic fragmentation pattern for 2,4-D ethyl ester included peaks at m/e 248, molecular ion; m/e 213, elimination of a chlorine atom; m/e 185, consecutive elimination of a chlorine atom and ethylene from the parent species; m/e 175, loss of the carboethoxy functional group from the molecular ion; and finally, a peak at m/e 162 presumably arising from a dichloro-

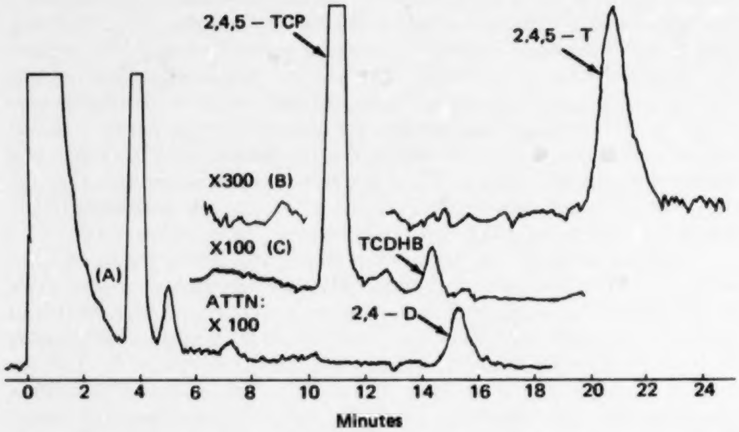


Fig. 2. Total ion current chromatograms of animal urine extracts containing (A) 2,4-D, (B) 2,4,5-T (chromatographed as ethyl esters), and (C) 2,4,5-trichlorophenol and an isomer of trichlorodihydroxybenzene (chromatographed as mono- and diethyl ethers); programmed temperature conditions: five min. at 180°C., to 220°C., at 5°C./min. isothermal at 220°C.

phenol or a dichlorocyclohexadienone radical ion. An analogous fragmentation scheme was observed for the ethyl ester of 2,4,5-T: m/e 282, molecular ion; m/e 247, loss of a chlorine atom; m/e 219, consecutive loss of a chlorine atom and ethylene from the parent ion; m/e 209, decomposition of the molecular ion with expulsion of the carboethoxyl function; and finally, the trichlorophenol or trichlorocyclohexadienone radical ion species at m/e 196. In addition to the intact residues of 2,4,5-T excreted in the urine, mass spectral evidence was obtained for the presence of the metabolites 2,4,5-trichlorophenol (TCP) and a trichlorodihydroxybenzene isomer observed as the mono- and diethyl ether derivatives, respectively. Thus, TCP ethyl ether displayed a molecular ion at m/e 224 and characteristic fragments of m/e 196 and m/e 160 due to consecutive loss of ethylene and HCl from the parent ion. The mass spectral evidence for the trichlorodihydroxybenzene diethyl ether included a molecular ion at m/e 268 and significant fragment ions at m/e 240, m/e 212, and m/e 176 arising from successive elimination of two molecules of ethylene and one molecule of HCl from the parent species.

In connection with the development of an analytical method for determination of organophosphate pesticides in human blood and urine, mass spectral confirmation of residues of several methylated and ethylated derivatives of the hydrolytic or metabolic products of organophosphorus insecticides was required. Utilizing a published analytical procedure (Shafik and Enos 1969) coupled with a silica gel column chromatographic cleanup (Shafik and Bradway 1970), extracts of human urine of an individual occupationally exposed to parathion were examined by gas chromatography-mass spectrometry. Programmed temperature analyses which were performed on a glass capillary column, 110 ft. × 0.025 in. i.d., coated with Versamid 900 containing five percent Igepal CO-880 resulted in the total ion monitor chromatogram shown in Figure 3. The metabolic and/or hydrolytic products of parathion excreted in the urine included both diethyl phosphoric acid and diethyl thiophosphoric acid either free or in salt form. Derivatization of these residues with diazomethane resulted in the formation of the three dialkyl phosphate compounds: DEMMTP, DEMMPTh, and DEMMP. Earlier (Shafik *et al.* 1970), it had been shown by combined gas chromatography-mass spectrometry and other analytical data that the later eluting major product (85 percent) of the methylation of diethyl thiophosphoric acid under the conditions of the analytical method was DEMMPTh and that the minor product (15 percent) was DEMMTP. Accordingly, all three trialkyl phosphates were observed and confirmed in the analysis of the human urine extract. Sufficient bond energy differences are associated with the isomeric structures DEMMPTh and DEMMTP that qualitatively and quantitatively dissimilar fragmentation modes are observed for the two isomers (Biros and Ross 1970).

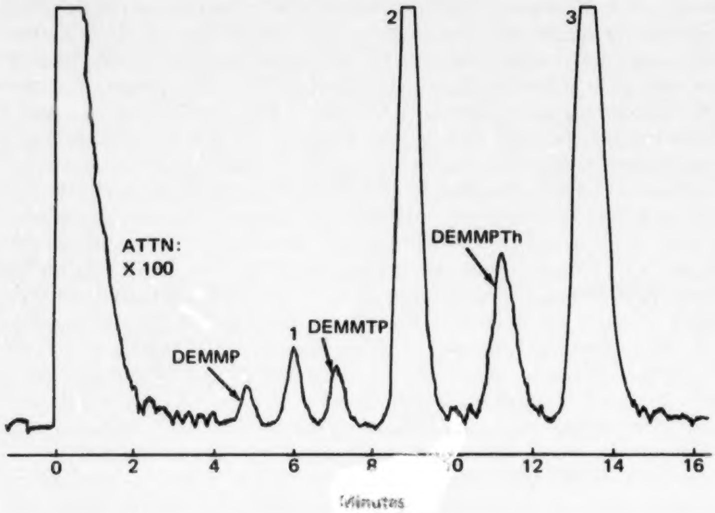


Fig. 3. Total ion current chromatogram of human urine extract containing methylated hydrolytic and metabolic products of parathion; programmed temperature conditions: five min. at 75°C., to 125°C. at 5°C./min., isothermal at 125°C.

Biros and Walker (1970) have described the application of combined gas chromatography-mass spectrometry to pesticide residue analysis in human tissue, specifically human adipose and liver tissue. A general, extensive extraction and cleanup procedure adapted from existing methods was used to isolate and purify the organochlorine insecticide residues.

Pesticides were extracted, subjected to liquid-liquid partitioning and Florisil column cleanup, and finally separated by a silica gel chromatographic step into two fractions with sufficient resolution on the gas chromatographic column for identification of seven pesticide residues from the adipose tissue sample and six pesticide residues from the liver tissue sample. These included β-HCH and γ-HCH, heptachlor epoxide, dieldrin, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT, and *p,p'*-DDT. Gas chromatographic separations were performed on a coiled glass column, 4 ft. × 1/8 in. o.d., packed with three percent OV-210 on 80/100 mesh Chromosorb W (HP).

Instrumental sensitivity was sufficient to identify residues at concentrations of 0.30 p.p.m. Computer enhancement techniques permitted identification of heptachlor epoxide residues at a level of 0.07 p.p.m. Definitive confirmation of pesticide residues was obtained by comparison of parent and fragment ion intensities and mass numbers of eluted residues and reference pesticides. Some difficulties were encountered in the absolute confirmation of configurational and structural isomers where mass spectral peak intensities and mass values of characteristic fragment ions were too similar under the conditions of the analysis. In those instances, however, identification was made readily on the basis of gas chromatographic retention times. The estimated lower limit of detectability of pesticide residues by the analytical scheme and instrument configuration reported was determined to be approximately 0.05 to 0.1 p.p.m.

Combined gas chromatography-mass spectrometry has been utilized by WIDMARK (1967) to provide confirmatory evidence for the presence of polychlorinated biphenyls (PCB) in fish, seabirds, conifer needles, and human depot fat. Later, BIROS *et al.* (1970) reported the mass spectral behavior of standards of PCB materials and PCB compounds isolated from human adipose tissue samples suspected to contain these residues. An example of the total ion monitor chromatogram obtained by programmed temperature analysis of a mixture of standard PCB compounds (Aroclor 1254) on a stainless steel capillary, 100 ft. × 0.02 in. i.d., coated with OV-1 silicone oil, is shown in Figure 4. Most components of the Aroclor standard produced mass spectra with molecular ion groups of high intensity. In addition, the characteristic isotopic distribution pattern corresponding to the number of chlorine atoms in the parent ion and chlorine-containing fragment ions was observed. Other noteworthy features of the mass spectra were the relatively intense fragmentation ions resulting from consecutive loss of chlorine atoms from the parent ion and the presence of intense doubly charged fragments within the mass spectra of most of the PCB compounds. Thus, it was possible to establish the molecular weight and number of chlorine atoms associated with each major numbered component in the chromatogram. The major components of the Aroclor 1254, on this basis, were shown to consist of at least two tetrachlorobiphenyl isomers, four pentachlorobiphenyl isomers, and five hexachlorobiphenyl compounds.

Although it was apparent that several of the major peaks in the chromatogram of the PCB materials were, in fact, composed of more than a single component, no attempt was made to elaborate further on the identity or nonidentity of the individual components of each of these peaks.

Detailed structural studies of each PCB component must necessarily involve the application of complex separation procedures, including ultra-high resolution gas chromatographic columns, thin-layer chromatography, and/or preparative gas chromatography, coupled with mass spectral ion intensity studies, infrared and nuclear magnetic resonance spectroscopy, and other spectroscopic techniques. Figure 4 also includes the total ion current monitor chromatogram of a human adipose tissue extract which was shown to contain traces of PCB

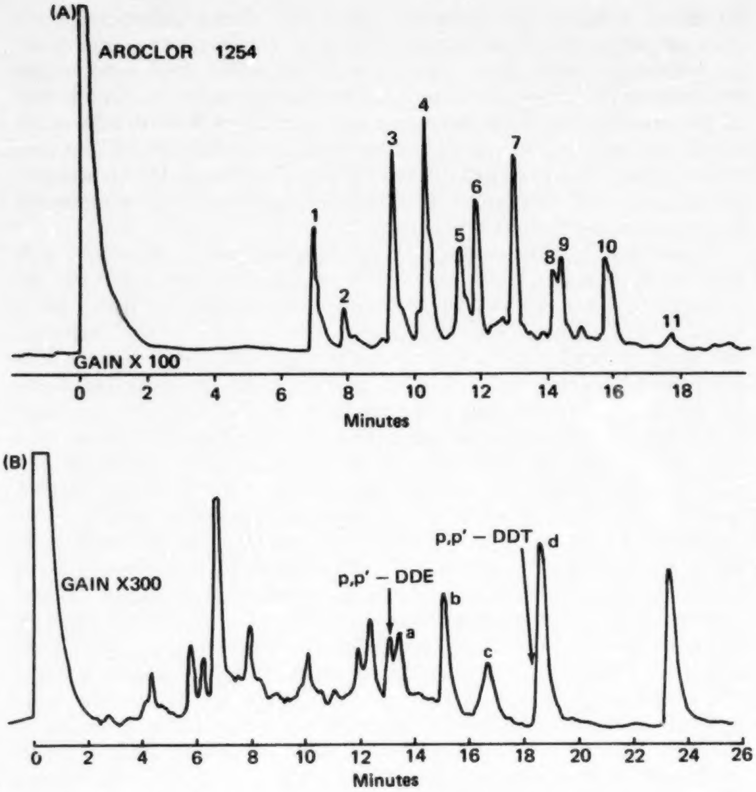


Fig. 4. Total ion current monitor chromatograms of (A) standard Aroclor 1254 mixture of polychlorinated biphenyls, programmed temperature analysis: two min. at 185°C., to 210°C. at 5°C./min., isothermal at 210°C.; and (B) human adipose tissue extract containing chlorinated pesticides and polychlorinated biphenyl materials, programmed temperature analysis: five min. at 180°C., to 210°C. at 5°C./min., isothermal at 210°C.

compounds. The mass spectra of peaks *a, b, c,* and *d* were consistent with polychlorinated biphenyl structures. Additional analytical data were reported by the authors for Aroclor 1260 PCB standard.

BAGLEY *et al.* (1970), utilizing combined gas chromatography-mass spectrometry in conjunction with thin-layer chromatographic separation of standard materials as well as tissue extracts, have identified PCB in bald eagle specimens collected in the field. They detected a total of 19 PCB compounds in the field samples, using a spiral glass chromatographic column (9 ft. × 0.25 in. o.d.) packed with one percent SE-30 on 100/120 mesh Gas Chrom Q. Mass spectra of the major components of Aroclor 1254 PCB standard are presented in this report as well as relative retention data of the individual PCB compounds.

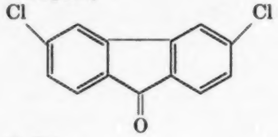
Studies of the conversion of aniline-type herbicides to azobenzenes in soil have been reported by KEARNEY *et al.* (1969), employing gas chromatographic-mass spectrometric analysis. Azobenzene condensation products resulting from incubation of soil samples with 3-chloroaniline, 3,4-dichloroaniline, and mixtures of the two compounds were analyzed using a 50-ft. support-coated open tubular column of 0.02 in. i.d. coated with SE-30 on Chromosorb W. Mixed chloroazobenzene formation was noted in the soil sample originally inoculated with both chloroanilines. Thus, the soil sample treated with 3-chloroaniline contained 3,3′-dichloroazobenzene (m/e 250, M+), the sample treated with 3,4-dichloroaniline contained 3,3′,4,4′-tetrachloroazobenzene (m/e 318, M+), and the sample treated with both chloroanilines contained, in addition to the symmetrical azobenzenes, a small quantity of 3,3′,4-trichloroazobenzene (m/e 284, M+). Mass spectra were compared with those obtained for available authentic samples.

In a study of the synthesis and reactions of a proposed DDT metabolite, 2,2-bis(*p*-chlorophenyl) acetaldehyde, reported by MCKINNEY *et al.* (1969), gas chromatography-mass spectrometry was used to characterize this synthetically prepared material. Treatment of 2,2-dichloro-1,1-bis(*p*-chlorophenyl) ethane with sodium hydroxide in refluxing ethylene glycol produced the cyclic acetal 2-(*p,p′*-dichlorobenzhydryl)-1,3-dioxolane whose structure was confirmed by NMR, infrared, and mass spectrometry. Subsequent hydrolysis of this acetal yielded two major components as indicated by both thin-layer and gas chromatography. The low-resolution mass spectrum of the later-eluting component showed it to be the desired aldehydic material (m/e 264, M+). Also found in the hydrolysate was a variable amount of *p,p′*-dichlorobenzophenone, confirmed by its mass spectrum (m/e 250, M+) when compared with an authentic sample.

The photochemical reactions of *p,p′*-DDE and *p,p′*-DDT in methanol in the presence of oxygen and nitrogen have been studied in detail by PLIMMER *et al.* (1970). Several significant photolysis products whose structures have been assigned on the basis of combined gas chromatographic-mass spectrometric data are given in Table III.

Some important photochemical reactions postulated and supported on the basis of the characterized products include photolytic generation of free radicals that may abstract hydrogen from solvent, react with oxygen, or abstract hydrogen from unreacted substrate. Further decomposition of short-lived intermediates yields many compounds. Oxidation products isolated include benzoic acids, aromatic ketones and polychlorinated phenols. *p,p′*-DDE also undergoes photocyclization to give a dichlorofluorene derivative. Mass spectral studies have been extended to model compounds, and the photochemistry of *p,p'*-dichlorobenzophenone has also been investigated to provide information on the further breakdown of photolytic products. Mass spectral data alone have been used to assign structures where trace quantities were obtained and authentic material was unavailable.

Table III. *Principal neutral photolysis products of p,p'-DDT and p,p'-DDE in methanol*
[From PLIMMER *et al.* (1970)]

	A. Photoconversion products of *p,p'*-DDT:	
m/e	Compound[a]	
222	R_2	(N_2)
250	R_2CO	(N_2)
260	$PhRCHCO_2CH_3$	(O_2)
284	$PhRCH{-}CH{-}Cl_2$	(O_2)
294	$R_2CHCO_2CH_3$	(O_2)
316	$R_2C{=}CCl_2$ (*p,p'*-DDE)	(N_2)
318	$R_2CH{-}CH{-}Cl_2$ (*p,p'*-DDD)	(O_2)
	B. Photoconversion products of *p,p'*-DDE:	
m/e	Compound[a]	
170	RCO_2CH_3	$(N_2;O_2)$
248		(O_2)
250	R_2CO	(N_2)
282	$R_2C{=}CHCl$	(N_2)
324	$R_2C(OCH_3)CO_2CH_3$	(O_2)
338	Unknown	(O_2)

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[a] R is ClC_6H_4; Ph is C_6H_5. The compounds listed represent the volatile components of the photolysate separated by gas chromatography. Presence of nitrogen (N_2) or oxygen (O_2) is indicated in parentheses following the compound.

VII. Conclusions

The application of mass spectrometry has continued to gain importance as an analytical technique for the characterization of pesticide residues and their metabolites, as well as other environmental chemical pollutants. The utility of the technique in structural elucidation studies of pesticidal metabolites and other chemical degradation products in animals, plants, and the environment has long been recognized and increased reliance is being placed on characteristic mass spectral fragmentation processes in this application. Although mass spectrometry should be employed as an extremely valuable adjunct to other instrumental techniques such as nuclear magnetic resonance, infrared, and ultraviolet spectrophotometry, of particular note in connection with the application of the technique to pesticide residues is the vast amount of structural information that can be obtained from a single mass spectrum. Thus, in many cases, because of the high sensitivity of this technique, definitive and incisive structural information can be obtained when the sample available to the analyst is truly limited.

In addition, combined gas chromatography-mass spectrometry affords a particularly useful approach to the analysis of pesticide residues because the components of a mixture can be positively identified without prior separation at sensitivities compatible with the limited quantities of residues encountered. From the results of recent studies involving the application of this technique, it has been demonstrated that available residue analytical methods or simple modifications of them provide efficient isolation and adequate cleanup of extracts of human, animal, and environmental media in most cases to permit gas chromatographic-mass spectrometric analysis with maximum confidence. Additionally, it has been shown that this combined technique will conveniently provide definitive and conclusive confirmation of residue identity, as well as characterization of residues and their metabolites of unknown structure.

From a technique standpoint, additional studies should be made on improvement of instrumental sensitivity to allow routine confirmation of pesticide residues at the nanogram and possible picogram level. Some gains in this area have already been reported from the use of computer enhancement techniques, pulse height analysis, and other methods. Studies are required for the documentation of compound yield and enrichment factors for the several classes of organic pesticides and various gas chromatographic-mass spectrometric interfacial systems under a variety of operating conditions. Technological studies designed to exploit systematically the advantages of capillary and other gas chromatographic columns for pesticide residue analyses are also needed. Finally, obvious advantages would accrue from the development of a complete residue analysis system, including an electron capture detector or flame ionization detector and splitter arrangement in tandem with the molecular separator permitting simultaneous mass spectrometric identification and gas chromatographic detection and quantitation of pesticide residues from a single gas chromatographic injection.

With the vast amounts of information generated during the course of combined gas chromatographic-mass spectrometric analysis of extracts of residue-containing substrates, the application of special purpose data acquisition systems (HITES and BIEMANN 1968), and computer searching systems (CRAWFORD and MORRISON 1968) would greatly alleviate the tedious task of data reduction for confirmation and structural characterization of pesticide residues.

The reported applications of combined gas chromatography-mass spectrometry to the analysis of pesticide residues have been increasing in frequency in recent years. With developments in instrument technology and availability of instrumentation it can be expected that the technique will be relied upon to an even greater extent in the future.

Acknowledgments

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The author also thanks H. L. Reynolds, *U. S. Food and Drug Administration* for reviewing the manuscript and making helpful suggestions regarding content and format.

Table IV. *Chemical designations of pesticides and related compounds mentioned in text.*

Pesticide	Chemical designation
Aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
Ametryne	2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-*s*-triazine
Amitrole	3-amino-*s*-triazole
Atrazine	2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine
Bayer 37344	4-(methylthio)-3,5-xylyl *N*-methylcarbamate
Bayer 44646	4-dimethylamino-*m*-tolyl *N*-methylcarbamate
Bromacil	5-bromo-3-*sec*-butyl-6-methyluracil
Carbaryl	1-naphthyl *N*-methylcarbamate
Chlordane	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene
Chlordene	4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Chlorobenzilate	ethyl 4,4′-dichlorobenzilate
CIPC	*m*-chloro-isopropylcarbanilate
2,4-D	2,4-dichlorophenoxyacetic acid
2,4-DB	α-(2,4-dichlorophenoxy) butyric acid
p,p′-DDA	*p,p′*-dichlorodiphenyl acetic acid

Table IV. (Continued)

Pesticide	Chemical designation
o,*p*'-DDD	1,1-dichloro-2-(*o*-chlorophenyl) 2-(*p*-chlorophenyl) ethane
p,*p*'-DDD	1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane
o,*p*'-DDE	1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethylene
p,*p*'-DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene
o,*p*'-DDT	1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane
p,*p*'-DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane
DDVP	2,2-dichlorovinyl dimethyl phosphate
Delnav	*p*-dioxane-2,3-diyl ethyl phosphorodithioate
DEMMP	*O*,*O*-diethyl-*O*-methyl phosphate
DEMMPTh	*O*,*O*-diethyl *S*-methyl phosphorothiolate
DEMMTP	*O*,*O*-diethyl *O*-methyl phosphorothionate
Diazinon	*O*,*O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate
Dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene
Dimetilan	1-(dimethylcarbamoyl)-5-methyl-3-pyrazolyl *N*,*N*-dimethylcarbamate
Dimite	4,4'-dichloro-α-methylbenzhydrol
Diphenamid	*N*,*N*-dimethyl-2,2-diphenyl acetamide
Diquat	1,1'-ethylene-2,2'-bipyridylium dibromide
Di-Syston	*O*,*O*-diethyl *S*-[(2-ethylthio) ethyl] phosphorodithioate
Diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea
Endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*endo*-5,8-dimethanonaphthalene
Eptam	ethyl *N*,*N*-dipropylthiolcarbamate
Ethion	*O*,*O*,*O*',*O*'-tetraethyl *S*,*S*'-methylenebisphosphorodithioate
Ethoxinol	1-ethoxymethyl-1,1-di(*p*-chlorophenyl) carbinol
Ferbam	ferric dimethyldithiocarbamate
Guthion	*O*,*O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl phosphorodithioate
Gutoxon	*O*,*O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl phosphorothiolate
β-HCH	β-isomer of 1,2,3,4,5,6-hexachlorocyclohexane
γ-HCH	γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane
Heptachlor	1,4,5,6,7,8,8a-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Heptachlor epoxide	1,4,5,6,7,8,8a-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindene
Imidan	*O*,*O*-dimethyl *S*-phthalimidomethyl phosphorodithioate
IPC	isopropylcarbanilate
Isodrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*,*endo*-5,8-dimethanonaphthalene
Isolan	1-isopropyl-3-methylpyrazolyl-5 *N*,*N*-dimethylcarbamate
Kelthane	4,4'-dichloro-α-(trichloromethyl) benzhydrol
Malaoxon	diethyl mercaptosuccinate, *S*-ester with *O*,*O*-dimethyl phosphorothiolate
Malathion	diethyl mercaptosuccinate, *S*-ester with *O*,*O*-dimethyl phosphorodithioate
Maneb	manganese ethylenebisdithiocarbamate
MCPA	4-chloro-2-methyl phenoxyacetic acid

Table IV. (Continued)

Pesticide	Chemical designation
MCPP	2-(2-methyl-4-chlorophenoxy) propionic acid
Methoxychlor	1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl) ethane
Methyl Trithion	*S*-(*p*-chlorophenylthio) methyl *O,O*-dimethyl phosphorodithioate
Nicotine	1-methyl-2-(3′-pyridyl)-pyrrolidine
Nonachlor	1,2,3,4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Parathion	*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate
Patoran	3-(*p*-bromophenyl)-1-methyl-1-methoxyurea
Perthane	1,1-dichloro-2,2-bis(*p*-ethylphenyl) ethane
Phosdrin	methyl 3-hydroxy-*alpha*-crotonate dimethyl phosphate, mixed isomers
Phosphamidon	2-chloro-2-dimethylcarbamoyl-1-methylvinyl dimethyl phosphate
Prometon	2-methoxy-4,6-bis(isopropylamino)-*s*-triazine
Prometryne	2-methylmercapto-4,6-bis(isopropylamino)-*s*-triazine
Propazine	2-chloro-4,6-bis(isopropylamino)-*s*-triazine
Pyramat	6-methyl-2-propyl-4-pyrimidinyl *N,N*-dimethylcarbamate
Siduron	1-(2-methylcyclohexyl)-3-phenylurea
Simetryne	2-methylmercapto-4,6-bis(ethylamino)-*s*-triazine
Systox thiolo	*O,O*-diethyl *S*[2-(ethylthio) ethyl] phosphorothioate
Systox thiono	*O,O*-diethyl *O*[2-(ethylthio) ethyl] phosphorothioate
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
Temik	2-methyl-2-(methylthio) propionaldehyde *O*-(methylcarbamoyl) oxime
Terbacil	3-*tert*-butyl-5-chloro-6-methyluracil
Trithion	*O,O*-diethyl *S*-[(*p*-chlorophenylthio) methyl] phosphorodithioate
Zineb	zinc ethylenebisdithiocarbamate
Ziram	zinc dimethyldithiocarbamate

Summary

Mass spectral fragmentation studies of several classes of organic pesticidal compounds have been outlined. These include the organochlorine and organophosphate classes of insecticides, carbamates, uracil, and triazine herbicides, and several miscellaneous classes including nicotinoids, rotenoids, and phenoxyalkanoic acids. The available literature relating to the application of mass spectrometry in the characterization of individual pesticide residues has been reviewed with emphasis on studies involving animal metabolism of pesticides, plant- and soil-induced chemical alteration of pesticidal materials, and the photodecomposition of pesticides. Techniques for the separation, isolation, and collection of individual pesticide residues for subsequent mass spectral analysis have been tabulated, with special refer-

ence to gas chromatographic and thin-layer chromatographic trapping devices.

The increasing literature availability of mass spectral correlations of pesticidal compounds has greatly facilitated the interpretation of the mass spectra of their unknown metabolites. Furthermore, current interest has focused on the relationships of factors which affect mass spectrometric and photochemical decomposition pathways since apparent analogies resulting in useful empirical correlations have been observed in several instances between unimolecular decomposition resulting from electron impact and nonionizing ultraviolet radiation. Increased emphasis is also being placed on the combined gas chromatographic-mass spectrometric technique for the analysis of pesticide residues, as it provides definitive structural characterization in a single instrumental approach without the necessity of resorting to the application of two or more supplementary analytical confirmatory procedures. A discussion is presented on the various types of coupling techniques used for interfacing a gas chromatograph with a mass spectrometer. Included are methods utilizing direct coupling of gas chromatographic columns as well as those employing carrier gas separator interfaces operating on diffusion or effusion principles. Advantages and disadvantages of each technique are indicated where this information is available. Other mass spectrometric instrumental considerations are also outlined, particularly those associated with enhancement of sensitivity, identification of multicomponent gas chromatographic peaks, and the use of mass spectrometry as a gas chromatographic detector in the confirmation of chemical identity. Recent applications of the combined technique to the confirmation of pesticide residues and the characterization of metabolites and photodecomposition products are discussed. Specific examples include the analysis of organophosphorus insecticides and metabolic products, 2,4-D and 2,4,5-T herbicides, organochlorine pesticides, polychlorinated biphenyls, phenolic pesticides or metabolic products, aniline type herbicides, and photodecomposition products of *p,p′*-DDT and *p,p′*-DDE.

Résumé*

Applications récentes de la spectrométrie de masse et du couple chromatographie gazeuse—spectrométrie de masse à l'analyse des résidus de pesticides

L'étude des spectres de masse de plusieurs pesticides organiques de différents groupes a été esquissée. Elle comprend les insecticides

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[*] Traduit par R. Mestres.

organochlorés et organophosphorés, les carbamates, les desherbants dérivés de l'uracile et de la triazine et plusieurs catégories diverses comprenant les dérivés de la nicotine, de la roténone et les acides phénoxy aliphatiques. La Littérature existante concernant l'application de la spectrométrie de masse à la caractérisation des résidus de pesticides a été passée en revue en soulignant les études intéressant le métabolisme chez l'animal les transformations chimipues provopuées par les plantes et le sol et la photo décomposition. Des techniques pour la séparation, l'isolement et la présentation des résidus de pesticides pour l'analyse ultérieure par spectrométrie de masse ont été répertoriées en se référant particulièrement aux systèmes de piégeage utilisés pour la chromatographie gazeuse et la chromatographie en couche mince.

La disposition de données toujours plus nombreuses sur les corrélations des spectres de masse des pesticides a grandement facilité l'interprétation des spectres de leurs métabolites inconnus. De plus, un intérêt général s'est concentré sur les similitudes des facteurs qui affectent les spectres de masse et les catabolismes photochimiques puisque des analogies apparentes conduisant à d'utiles corrélations empiriques ont été observées dans plusieurs cas entre la décomposition mono moléculaire dûe à un impact d'électron et les radiations ultraviolettes non ionisantes. L'accent a aussi été mis sur le couplage avec la chromatographie gazeuse pour l'analyse des résidus de pesticides qui fournit une caractérisation de structure définitive au moyen d'une seule méthode instrumentale sans le recours nécessaire d'une confirmation par deux ou plusieurs méthodes analytiques supplémentaires. Les divers modes de couplage du chromatographe et d'un spectromètre de masse sont présentés. Les méthodes réalisant le couplage direct des colonnes chromatographiques ainsi que celles utilisant des interfaces obtenant la séparation du gaz vecteur par diffusion ou effusion sont décrites. Les avantages et les inconvénients de chaque technique sont indiqués lorsque des informations existent à leur sujet.

D'autres considérations sur la spectrométrie de masse sont aussi traitées, en particulier celles concernant l'augmentation de sensibilité, l'identification de pics de plusieurs composés non résolus en chromatographie et l'emploi de la spectrométrie de masse comme détecteur de chromatographie gazeuse pour la confirmation de l'identité chimique.

Des applications récentes de cette technique pour la confirmation des résidus de pesticides et la caractérisation des métabolites et des produits de photodécomposition sont décrites.

Les exemples spécifiques comprennent l'analyse des insecticides organophosphorés et leurs métabolites, les desherbants 2,4-D et 2,4,5-T, les pesticides organochlorés, les biphényles chlorés, les pesticides phénoliques ou leurs métabolites, les desherbants du type aniline et enfin les produits de photodécomposition du *p,p'*-DDT et du *p,p'*-DDE.

Zusammenfassung*

Neuere Anwendungen der Massenspektrometrie und der kombinierten Gaschromatographie/Massenspektrometrie bei der Analyse von Pestizid-Rückständen

Massenspektrometrische Untersuchungen von Bruchstücken aus verschiedenen Gruppen von organischen Pflanzenschutzmitteln wurden umrissen. Sie umfassen die Gruppen der Organochlor- und Organophosphor-Insektizide, der Carbamate, der Uracil- und Triazin-Herbizide, und verschiedene andere Gruppen, wie z.B. Nicotinoide, Rotenoide und Phenoxyalkan-Säuren. Die verfügbare Literatur über die Anwendung der Massenspektrometrie zur Charakterisierung einzelner Pestizid-Rückstände wurde besonders im Hinblick auf solche Arbeiten ausgewertet, die sich mit dem tierischen Stoffwechsel von Pestiziden, den durch Pflanzen und Boden induzierten chemischen Umwandlungen von Pflanzenschutzmitteln, und dem photochemischen Abbau von Pestiziden befassen. Verfahren zur Trennung, zur Isolierung und zur Anreicherung einzelner Pestizid-Rückstände zwecks anschließender massenspektroskopischer Analyse wurden tabellarisch dargestellt, und zwar unter besonderer Berücksichtigung von gas- und dünnschicht-chromatographischen Auffangvorrichtungen.

Die anwachsende Literatur über massenspektrometrische Korrelationen pestizider Verbindungen hat die Interpretation der Massenspektren ihrer unbekannten Metaboliten beträchtlich erleichtert. Außerdem hat sich ein akutes Interesse auf die Beziehungen zwischen solchen Faktoren konzentriert, die massenspektrometrische und photochemische Abbauwege beeinflussen, seit nämlich offensichtliche Analogien beobachtet wurden, die zu brauchbaren empirischen Korrelationen führten; dies betrifft verschiedene Fälle von Analogien zwischen monomolekularem Zerfall als Folge von Elektronenstößen und einem solchen aufgrund nicht-ionisierender ultravioletter Strahlung. Besonderer Nachdruck wird auch auf das kombinierte Gaschromatographie/Massenspektrometrie-Verfahren zur Analyse von Pestizid-Rückständen gelegt, mit dessen Hilfe definitive Strukturcharakterisierungen in einem einzigen instrumentellen Schritt möglich waren, ohne zu einem Einsatz von zwei oder mehr zusätzlichen analytischen Bestätigungsverfahren Zuflucht nehmen zu müssen. Die verschiedenen Arten von Kombinationsverfahren zur Kopplung zwischen Gaschromatograph und Massenspektrometer werden diskutiert. Eingeschlossen sind Verfahren, bei denen der Anschluß unmittelbar an die gaschromatographischen Säulen erfolgt, ebenso wie auch solche, bei denen Trägergas-Separatoren auf Diffusions- oder Effusionsbasis zwischengeschaltet sind. Die Vor- und Nachteile

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* Übersetzt von H. Frehse.

der einzelnen Verfahren werden aufgezeigt, soweit Informationen darüber vorliegen.

Andere gerätetechnische Betrachtungen bezüglich der Massenspektrometrie werden ebenfalls umrissen, besonders diejenigen, die mit einer Steigerung der Empfindlichkeit, der Identifizierung gaschromatographischer Multikomponenten-Peaks, und der Anwendung der Massenspektrometrie als gaschromatographischer Detektor bei der Bestätigung der chemischen Identität in Zusammenhang stehen. Neuere Anwendungen der kombinierten Verfahren zur Bestätigung von Pestizid-Rückständen und zur Charakterisierung von Metaboliten und photochemischen Abbauprodukten werden diskutiert. Spezielle Beispiele umfassen die Analyse von Organophosphor-Insektiziden und -Metaboliten, 2,4-D- und 2,4,5-T-Herbiziden, Organochlor-Pestiziden, polychlorierten Biphenylen, phenolischen Pestiziden oder Metaboliten, Herbiziden auf Anilinbasis, und schließlich photochemischen Abbauprodukten von *p,p'*-DDT und *p,p'*-DDE.

References

Abbott, D. C., and J. Thomson: The application of thin-layer chromatographic techniques to the analysis of pesticide residues. Residue Reviews **11**, 1 (1965).

Amy, J. W., E. M. Chait, W. E. Baitinger, and F. W. McLafferty: A general technique for collecting gas chromatographic fractions for introduction into the mass spectrometer. Anal. Chem. **37**, 1265 (1965).

Bafus, D. A., E. J. Gallegos, and R. W. Kiser: An electron impact investigation of some alkyl phosphate esters. J. Phys. Chem. **70**, 2614 (1966).

Bagley, G. E., W. L. Reichel, and E. Cromartie: Identification of polychlorinated biphenyls in two bald eagles by combined gas chromatography-mass spectrometry. J. Assoc. Official Anal. Chemists **53**, 251 (1970).

Baldwin, M. K., and J. Robinson: Metabolism in the rat of the photoisomerization product of dieldrin. Nature **224**, 283 (1969).

Baron, R. L., J. A. Sphon, J. T. Chen, E. Lustig, J. D. Doherty, E. A. Hansen, and S. M. Kolbye: Confirmatory isolation and identification of a metabolite of carbaryl in urine and milk. J. Agr. Food Chem. **17**, 883 (1969).

Barthel, W. F., A. Curley, C. L. Thrasher, and V. A. Sedlak: Determination of pentachlorophenol in blood, urine, tissue and clothing. J. Assoc. Official Anal. Chemists **52**, 294 (1969).

Belasco, I. J., and R. W. Reiser: Metabolic fate of sodium in the animal. J. Agr. Food Chem. **17**, 1000 (1969).

Bellman, S. W., and T. L. Barry: Private communication, Food and Drug Administration *U.S. Department of Health, Education, and Welfare* (1970).

Benson, W. R., and J. N. Damico: Mass spectra of some carbamates and related ureas. II. J. Assoc. Official Anal. Chemists **51**, 347 (1968).

Bergstedt, L., and G. Widmark: Repetitive scanning in gas chromatography-mass spectrometry. Chromatographia **3**, 59 (1970).

Beynon, J. H.: Mass spectrometry and its applications to organic chemistry. 3rd ed. Amsterdam: Elsevier (1960).

Biemann, K.: Mass spectrometry: Organic chemical applications. 1st ed. New York: McGraw-Hill (1962).

Bierl, B. A., M. Beroza, and J. M. Ruth: Collection and transfer device for gas chromatographic fractions. J. Gas Chromatog. **6**, 286 (1968).

Biros, F. J.: Enhancement of mass spectral data by means of a time averaging computer. Anal. Chem. **42**, 537 (1970 a).

——— Applications of combined gas chromtography-mass spectrometry to pesticide residue identification. Presented Amer. Chem. Soc.-Chem. Inst. Canada Internat. Meeting, Toronto, May (1970 b).

———, and R. T. Ross: Fragmentation processes in the mass spectra of trialkylphosphates, phosphorothionates, phosphorothiolates, and phosphorodithioates. Presented 18th Conference on Mass Spectrometry and Allied Topics, San Francisco, June (1970).

———, and H. Sullivan: Unpublished observations (1970).

———, and A. C. Walker: Pesticide residue analysis in human tissue by combined gas chromatography-mass spectrometry. J. Agr. Food Chem. **18**, 425 (1970).

——— ———, and A. Medbery: Polychlorinated biphenyls in human adipose tissue. Bull. Environ. Contam. Toxicol. **5**, 317 (1970).

Black, D. R., R. A. Flath, and R. Teranishi: Membrane molecular separators for gas chromtographic-mass spectrometric interfaces. J. Chromatog. Sci. **7**, 284 (1969).

Blumer, M.: An integrated gas chromatograph-mass spectrometer system with carrier gas separator. Anal. Chem. **40**, 1590 (1968).

Budzikiewicz, H., C. Djerassi, and D. H. Williams: Mass spectrometry of organic compounds. 2nd ed. San Francisco: Holden-Day (1964).

Burson, K. R., and C. T. Kenner: A simple trap for collecting gas chromatographic fractions for mass spectrometer analysis. J. Chromatog. Sci. **7**, 63 (1969).

Cartwright, M., and A. Heywood: The use of molecular sieve 5A for collecting fractions from a gas chromatograph. Analyst **91**, 337 (1966).

Cavanagh, L. A.: Mass spectrometry of pesticides. S.R.I. Pesticide Research Bull. 3 (1), 1 (1963).

Cooks, R. G.: Bond formation upon electron impact. Org. Mass Spectrom. **2**, 481 (1969).

———, and A. F. Gerrard: Electron impact induced rearrangements in compounds having the P=S bond. J. Chem. Soc. (B), **1968**, 1327.

Crawford, L. R., and J. D. Morrison: Computer methods in analytical mass spectrometry. Identification of an unknown compound in a catalog. Anal. Chem. **40**, 1464 (1968).

Damico, J. N.: The mass spectra of some organophosphorus pesticide compounds. J. Assoc. Official Anal. Chemists **49**, 1027 (1966).

———, R. P. Barron, and J. M. Ruth: The mass spectra of some chlorinated pesticidal compounds. Org. Mass Spectrom. **1**, 331 (1968).

——— ———, and J. A. Sphon: Field ionization spectra of some pesticides and other biologically significant compounds. J. Mass Spectrom. Ion Phys. **2**, 161 (1969).

———, and W. R. Benson: The mass spectra of some carbamate pesticides. J. Assoc. Official Anal. Chemists **48**, 344 (1965).

———, J. T. Chen, C. E. Costello, and E. O. Haenni: Structure of Klein's metabolites of aldrin and dieldrin. J. Assoc. Official Anal. Chemists **51**, 48 (1968).

———, N. P. Wong, and J. A. Sphon: Use of activated charcoal to trap gas chromatographic fractions for mass spectrometric analysis and to introduce volatile compounds into the mass spectrometer. Anal. Chem. **39**, 1045 (1967).

Deverse, F. T., E. Gipstein, and L. G. Lesoine: Rapid identification of thin layer separations by mass spectrometry. Instrument News (Perkin-Elmer Corp.) **18** (2), 16 (1967).

Elgar, K. E.: Confirmation of identity. J. Assoc. Official Anal. Chemists **50**, 1069 (1967).

Fales, H. M., G. W. A. Milne, and M. L. Vestal: Chemical ionization mass spectrometry of complex molecules. J. Amer. Chem. Soc. **91**, 3682 (1969).

Feil, V. J., R. D. Hedde, R. G. Zaylskie, and C. H. Zachrison: Dieldrin-^{14}C metabolism in sheep. J. Agr. Food Chem. **18**, 120 (1970).

Flath, R. A., D. R. Black, R. R. Forrey, G. M. McDonald, T. R. Mon, and R. Teranishi: Volatiles in gravenstein apple essence identified by gc-mass spectrometry. J. Chromatog. Sci. **7**, 508 (1969).

Gardiner, J. A., R. W. Reiser, and H. Sherman: Identification of the metabolites of bromacil in rat urine. J. Agr. Food Chem. **17**, 967 (1969).

Gardner, A. M., J. N. Damico, E. A. Hansen, E. Lustig, and R. W. Storherr: Previously unreported homolog of malathion found as residue on crops. J. Agr. Food Chem. **17**, 1181 (1969).

Gibson, D. T., J. R. Koch, C. L. Schuld, and R. E. Kallio: Oxidation degradation of aromatic hydrocarbons by microorganisms. II. Metabolism of halogenated aromatic hydrocarbons. Biochem. **7**, 3795 (1968).

Gohlke, R. S.: Time of flight mass spectrometry and gas-liquid partition chromatography. Anal. Chem. **31**, 535 (1959).

Grayson, M. A., and C. J. Wolf: Efficiency of molecular separators for interfacing a gas chromatograph with a mass spectrometer. Anal. Chem. **39**, 1438 (1967).

——— ——— Two stage molecular separator for connecting a gas chromatograph to a mass spectrometer. Anal. Chem. **42**, 426 (1970).

Gunther, F. A.: Instrumentation in pesticide residue determination. Adv. Pest Control Research **5**, 191 (1962).

Hammar, C. G., B. Holmstedt, and R. Ryhage: Mass fragmentography. Identification of chlorpromazine and its metabolites in human blood by a new method. Anal. Biochem. **25**, 532 (1968).

Henderson, G. L., and D. G. Crosby: Photodecomposition of dieldrin and aldrin. J. Agr. Food Chem. **15**, 888 (1967).

Hites, R. A., and K. Biemann: Mass spectrometer computer system particularly suited for gas chromatography of complex mixtures. Anal. Chem. **40**, 1217 (1968).

Joly, D.: Identification and determination of traces by gas chromatography-mass spectrometry. Z. anal. Chem. **236**, 259 (1968).

Jorg, J., R. Houriet, and G. Spiteller: Massenspektren von Pflanzenschutzmitteln. Monatsh. für Chem. **97**, 1064 (1966).

Kabot, F.: How to collect fractions from the model 800 series gas chromatographs. GC Newsletter (Perkin-Elmer Corp.) 3 (1), 1 (1967).

Kantner, T. R., and R. O. Mumma: Application of mass spectroscopy to pesticide residue analysis. Residue Reviews **16**, 138 (1966).

Kearney, P. C., J. R. Plimmer, and F. B. Guardia: Mixed chloroazobenzene formation in soil. J. Agr. Food Chem. **17**, 1418 (1969).

Kennett, B. H.: Ratio recording of mass spectra in combined gas chromatography-mass spectrometry. Anal. Chem. **39**, 1506 (1967).

Krueger, P. M., and J. A. McCloskey: Porous stainless steel as a carrier gas separator interface material for gas chromatography-mass spectrometry. Anal. Chem. **41**, 1930 (1969).

Lawrence, J. H., R. P. Barron, J. T. Chen, P. Lombardo, and W. R. Benson: Note on identification of a chlordane metabolite in milk and cheese. J. Assoc. Official Anal. Chemists **53**, 261 (1970).

Leemans, F. A. J. M., and J. A. McCloskey: Combination gas chromatography-mass spectrometry. J. Amer. Oil Chem. Soc. **44**, 11 (1967).

Lewis, C. P.: Mass spectra of ethyl-*N*-phenylcarbamate and ethyl-*N*-ethyl carbamate. Anal. Chem. **36**, 176 (1964 a).

——— Mass spectra of *N*-substituted ethyl carbamates. Anal. Chem. 36, 1582 (1964 b).

Lipsky, S. R., C. G. Horvath, and W. J. McMurray: Utilization of system employing the selective permeation of helium through a unique membrane of Teflon as an interface for gas chromatograph and mass spectrometer. Anal. Chem. 38, 1585 (1966).

Littlewood, A. B.: The coupling of gas chromatography with methods of identification. I. Mass spectrometry. Chromatographia 1, 37 (1968).

Llewellyn, P., and D. Littlejohn: A molecular separator for gas chromatograph and mass spectrometer. Presented Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Feb. (1966).

Lovins, R. E.: Identification of pesticides in mixtures by high-resolution mass spectrometry. J. Agr. Food Chem. 17, 663 (1969).

Matsumura, F., G. M. Boush, and A. Tai: Breakdown of dieldrin in the soil by a micro-organism. Nature 219, 965 (1968).

McFadden, W. H.: Introduction of gas-chromatographic samples to a mass spectrometer. Sep. Science 1, 723 (1966).

———, and E. A. Day: Scan rate considerations in combined gas chromatography-mass spectrometry. Anal. Chem. 36, 2362 (1964).

McKinney, J. D., E. L. Boozer, H. P. Hopkins, and J. E. Suggs: Synthesis and reactions of a proposed DDT metabolite, 2,2-bis (*p*-chlorophenyl) acetaldehyde. Experientia 25, 897 (1969).

McLafferty, F. W.: Mass spectrometric analysis: Broad applicability to chemical research. Anal. Chem. 28, 306 (1956).

——— Mass spectrometry of organic ions. 1st ed. New York-London: Academic Press (1963).

Merritt, C., M. L. Bazinet, and W. G. Yeomans: Consideration of parameters for coupling gas chromatographs to mass spectrometers. J. Chromatog. Sci. 7, 122 (1969).

———, P. Issenberg, M. L. Bazinet, B. N. Green, T. O. Merron, and J. G. Murray: Fast scanning of high resolution mass spectra. Anal. Chem. 37, 1037 (1965).

Miller, D. O.: A high-temperature inlet manifold for coupling a gas chromatograph to a time-of-flight mass spectrometer. Anal. Chem. 35, 2033 (1963).

Morley, H. V.: Absorbents and their application to column cleanup of pesticide residues. Residue Reviews 16, 1 (1966).

Mumma, R. O., and T. R. Kantner: Identification of halogenated pesticides by mass spectroscopy. J. Econ. Entomol. 59, 491 (1966).

———, W. B. Wheeler, D. E. H. Frear, and R. H. Hamilton: Dieldrin: Extraction of accumulations by root uptake. Science 152, 530 (1966).

Odland, R. K., E. Glock, and N. L. Bodenhamer: A simple technique for trapping gas chromatographic samples from a capillary column for mass spectrometry or rechromatography on another column. J. Chromatog. Sci. 7, 187 (1969).

Onley, J. H., G. Yip, and M. H. Aldridge: A metabolic study of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) applied to corn seedlings. J. Agr. Food Chem. 16, 426 (1968).

Paulson, G. D., R. G. Zaylskie, M. V. Zehr, C. E. Portnoy, and V. J. Feil: Metabolites of carbaryl (1-naphthyl methylcarbamate) in chicken urine. J. Agr. Food Chem. 18, 110 (1970).

Plimmer, J. R., P. C. Kearney, and U. I. Klingebiel: Photochemical desulfurization of methyl thio-*s*-triazines. Tetrahedron Letters 44, 3891 (1969).

——— ———, D. D. Kaufman, and F. B. Guardia: Amitrole decomposition by free radical-generating systems and by soils. J. Agr. Food Chem. 15, 996 (1967).

———, U. I. Klingebiel, and B. E. Hummer: Photooxidation of DDT and DDE. Science **167,** 67 (1970).

Quayle, A: The mass spectra of some organic phosphates. In J. D. Waldron (ed.): Advances in mass spectrometry, p. 365. New York-London: Pergamon (1959).

Reed, R. I., and J. M. Wilson: Electron impact and molecular dissociation. XII. The cracking patterns of some rotenoids and flavones. J. Chem. Soc. **1963,** 5949.

Rees, D. I.: Interfacial systems for the coupling of gas chromatography with mass spectrometry. Talanta **16,** 903 (1969).

Reiser, R. W.: Mass spectra of substituted uracils. Org. Mass Spectrom. **2,** 467 (1969).

Rhodes, R. C., R. W. Reiser, J. A. Gardiner, and H. Sherman: Identification of the metabolites of terbacil in dog urine. J. Agr. Food Chem. **17,** 974 (1969).

Richardson, A., M. Baldwin, and J. Robinson: Identification of metabolites of dieldrin (HEOD) in the faeces and urine of rats. J. Sci. Food Agr. **19,** 524 (1968 a).

——— ——— ——— Metabolites of dieldrin (HEOD) in the urine and faeces of rats. Chem. Ind. (London), p. 588 (1968 b).

Rix, M. J., B. R. Webster, and I. C. Wright: A technique for obtaining the mass spectra of substances isolated by thin layer chromatography. Chem. Ind. (London), p. 452 (1969).

Robinson, J., A. Richardson, B. Bush, and K. E. Elgar: A photoisomerization product of dieldrin. Bull. Environ. Contamination Toxicol. **1,** 127 (1966).

Rosen, J. D.: The photolysis of diphenamid. Bull. Environ. Contam. Toxicol. **2,** 349 (1967).

———, and R. F. Strusz: Photolysis of 3-(*p*-bromophenyl)-1-methoxy-1-methylurea. J. Agr. Food Chem. **16,** 568 (1968).

——— ———, and C. C. Still: Photolysis of phenylurea herbicides. J. Agr. Food Chem. **17,** 206 (1969).

———, J. Sutherland, and G. R. Lipton: The photochemical isomerization of dieldrin and endrin and effects on toxicity. Bull. Environ. Contam. Toxicol. **1,** 133 (1966).

Ryhage, R.: Use of a mass spectrometer as a detector and analyzer for effluents emerging from high temperature gas liquid chromatography columns. Anal. Chem. **36,** 759 (1964).

Scanlan, R. A., R. G. Arnold, and R. C. Lindsay: Collecting and transferring packed column gas chromatographic fractions to capillary columns for fast scan mass spectral analysis. J. Gas Chromatog. **6,** 372 (1968).

Schechter, M. S.: The need for confirmation. Pest. Monitoring J. **2,** 1 (1968).

Scott, R. P. W., I. A. Fowlis, D. Welte, and T. Wilkins: Interrupted-elution gas chromatography. In A. B. Littlewood (ed.): Gas chromatography, p. 318. Surrey, Eng.: Bartholomew Press (1967).

Sellier, N., and G. Viochron: Influence of gc-ms coupling on the performances of a chromatographic column. J. Chromatog. Sci. **8,** 147 (1970).

Shafik, M. T., and D. Bradway: A cleanup procedure for the determination of low levels of alkyl phosphates, thiophosphates, and dithiophosphates in rat urine. Presented Amer. Chem. Soc.—Chem. Inst. Canada Internat. Meeting, Toronto, May (1970).

——— ———, F. J. Biros, and H. F. Enos: Characterization of the alkylation products of diethyl phosphorothioate. J. Agr. Food Chem. **18,** 1174 (1970).

———, and H. F. Enos: Determination of the metabolic and hydrolytic products of organophosphorus pesticide chemicals in human blood and urine. J. Agr. Food Chem. **17,** 1186 (1969).

———, and H. Sullivan: A method for the determination of low level exposure to 2,4-D and 2,4,5-T. Presented 7th Inter-Amer. Conference on Toxic. and Occupat. Med., Miami, Aug. (1970).

Slade, P., and A. E. Smith: Photochemical degradation of diquat. Nature **213,** 919 (1967).

Sphon, J. A., and J. N. Damico: The mass spectra of some chlorinated aromatic pesticidal compounds. Org. Mass Spectrom. **3,** 51 (1970).

Sweeley, C. C., W. H. Elliott, I. Fries, and R. Ryhage: Mass spectrometric determination of unresolved components in gas chromatographic effluents. Anal. Chem. **38,** 1549 (1966).

Thomson, J. B., P. Brown, and C. Djerassi: Mass spectrometry in structural and stereochemical problems. CXIV. Electron impact induced rearrangement of thiocarbonates, carbamates, and thiocarbamates. J. Amer. Chem. Soc. **88,** 4049 (1966).

Turro, N. J., D. C. Neckers, P. A. Leermakers, D. Seldner, and P. D'Angelo: Photochemistry of organic molecules. I. Ketene dimers and other small ring compounds. J. Amer. Chem. Soc. **87,** 4097 (1965).

Watkins, D. A. M., and D. Woodcock: Photochemical decomposition of 1-naphthalene acetic acid. Chem. Ind. (London), p. 1522 (1968).

Watson, J. T., and K. Biemann: Direct recording of high resolution mass spectra of gas chromatographic effluents. Anal. Chem. **37,** 844 (1965).

Westlake, W. E., and F. A. Gunther: Advances in gas chromatographic detectors illustrated from applications to pesticide residue evaluations. Residue Reviews **18,** 175 (1967).

Wheeler, W. B., D. E. H. Frear, R. O. Mumma, R. H. Hamilton, and R. C. Cotner: Quantitative extraction of root absorbed dieldrin from the aerial parts of forage crops. J. Agr. Food Chem. **15,** 227 (1967).

Widmark, G.: Possible interference by chlorinated biphenyls. J. Assoc. Official Anal. Chemists **50,** 1069 (1967).

———, L. Bergstedt, and A. Laveskog: Mass spectrometry of organochlorine compounds and attempts to reach positive identification. Presented Applied Chem. Div. I.U.P.A.C. Conference, Sittingbourne, Kent, U.K., Oct. (1968).

Willis, D. E.: Trapping technique for open tubular chromatographic columns. Anal. Chem. **40,** 1597 (1968).

Wooley, W. D.: An improved technique for transferring fractions from a gas chromatograph to a mass spectrometer. Analyst **94,** 121 (1969).

Recent references added in proof and not cited in text

Baldwin, M. K., J. Robinson, and D. V. Parke: Metabolism of endrin in the rat. J. Agr. Food Chem. **18,** 1117 (1970).

Bartley, W. J., N. R. Andrawes, E. L. Chancey, W. P. Bagley, and H. W. Spurr: The metabolism of Temik aldicarb pesticide [2-methyl-2-(methylthio) propionaldehyde O-(methylcarbamoyl) oxime] in the cotton plant. J. Agr. Food Chem. **18,** 446 (1970).

Bonelli, E. J.: Computer-controlled GC/MS for the analysis of polychlorinated biphenyls. Amer. Lab. **3,** 27 (1971).

Boulton, A. A., and J. R. Majer: Mass spectrometry of crude biological extracts. Absolute quantitative detection of metabolites at the submicrogram level. J. Chromatog. **48,** 322 (1970).

Briggs, G. G., and J. E. Dawson: Hydrolysis of 2,6-dichlorobenzonitrile in soils. J. Agr. Food Chem. **18,** 97 (1970).

Clemett, C. J.: Simple device for transferring thin-layer chromatographic fractions for spectroscopic examination. Anal. Chem. **43,** 490 (1971).

Curley, A., and R. Hawk: Mass spectra of isomeric 1,2,3,4,5,6-hexachlorocyclohexanes. Presented 160th Amer. Chem. Soc. Meeting, Chicago, Ill. (Sept. 1970.)

Dougherty, R. C.: A perturbation molecular orbital approach to the interpretation of organic mass spectra. The relationship between mass spectrometric, thermolytic, and photolytic fragmentation reactions. J. Amer. Chem. Soc. **90,** 5780 (1968 a).

——— Application of the pertubation molecular orbital method to the interpretation of organic mass spectra. The hexahelicine rearrangement and other electrocyclic mass spectrometric reactions. J. Amer. Chem. Soc. **90,** 5788 (1968 b).

Durden, J. A., Jr., H. W. Stollings, J. E. Casida, and M. Slade: The synthesis of hydroxymethylcarbamates. J. Agr. Food Chem. **18,** 459 (1970).

Gaffney, T. E., C. G. Hammar, B. Holmstedt, and R. E. McMahon: Ion specific detection of internal standards labelled with stable isotopes. Anal. Chem. **43,** 307 (1971).

Hammar, C. G., and R. Hessling: Novel peak matching technique by menas of a new and combined multiple ion detector-peak matcher device. Elemental analyses of compounds in submicrogram quantities without prior isolation. Anal. Chem. **43,** 298 (1971).

Hawes, J. E., R. Mallaby, and V. P. Williams: An all-glass in line temperature programmed interface for use in gas chromatograph-mass spectrometer combinations. J. Chromatog. Sci. **7,** 690 (1969).

Hutson, D. H., E. C. Hoadley, M. H. Griffiths, and C. Donninger: Mercapturic acid formation in the metabolism of 2-chloro-4-ethylamino-6-(1-methyl-1-cyanoethylamino)-s-triazine in the rat. J. Agr. Food Chem. **18,** 507 (1970).

Hutzinger, O., and W. D. Jamieson: Identification of organochlorine pesticides in crude extracts by mass spectrometry. Bull. Environ. Contamination Toxicol. **5,** 587 (1971).

——— ———, and S. Safe: Mass spectra of fifteen chlorinated aromatic fungicides. J. Assoc. Official Anal. Chemists **54,** 178 (1971).

——— ———, and V. Zitko: Identification of polychlorinated biphenyls and DDT in mixtures by mass spectrometry. Nature **226,** 664 (1970).

Jörg, J., M. Spiteller-Friedmann, and G. Spiteller: Applicability of mass spectrometric methods in residue analysis. Pflanzenschutzberichte **36,** 157 (1967); through Chem. Abstr., **68,** 86180 (1968).

Koemann, J. H., M. C. Ten Noever De Brauw, and R. H. De Vos: Chlorinated biphenyls in fish, mussels, and birds from the river Rhine and The Netherlands coastal area. Nature **221,** 1126 (1969).

Lamoureux, G. L., R. H. Shimabukuro, H. R. Swanson, and D. S. Frear: Metabolism of 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (atrazine) in excised sorghum leaf sections. J. Agr. Food Chem. **18,** 81 (1970).

Markey, S. P.: Improved glass frit interface for combined gas chromatography-mass spectrometry. Anal. Chem. **42,** 306 (1970).

Matsumura, F., and J. O. Nelson: Identification of the major metabolic product of heptachlor epoxide in rat feces. Bull. Environ. Contam. Toxicol. **5,** 489 (1971).

Matthews, H. B., and F. Matsumura: Metabolic fate of dieldrin in the rat. J. Agr. Food Chem. **17,** 845 (1969).

McGuire, R. R., M. J. Zabik, R. D. Schuetz, and R. D. Flotard: Photochemistry of bioactive compounds. Photolysis of 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene (cage formation vs photodechlorination). J. Agr. Food Chem. **18,** 319 (1970).

McMurray, W. J., S. R. Lipsky, C. Zioudrou, and G. L. Schmir: The mass spectra of N-phenylphosphoramidate esters. Org. Mass Spectrom. **3,** 1031 (1970).

Mücke, W., K. O. Alt, and H. O. Esser: Degradation of ^{14}C-labeled diazinon in the rat. J. Agr. Food Chem. **18,** 208 (1970).

Pape, B. E., M. F. Para, and M. J. Zabik: Photochemistry of bioactive compounds. Photodecomposition of 2-(1,3-dioxolane-2-yl)-phenyl-N-methyl carbamate. J. Agr. Food Chem. 18, 490 (1970).

——— ———, and M. J. Zabik: Photochemistry of bioactive compounds. Photochemistry of selected 2-chloro- and 2-methylthio-4,6,-di(alkylamino)-s-triazine herbicides. J. Agr. Food Chem. 18, 202 (1970).

Pardue, J. R., E. A. Hansen, R. P. Barron, and J. T. Chen: Diazinon residues on field-sprayed kale. Hydroxydiazinon—a new alteration product of diazinon. J. Agr. Food Chem. 18, 405 (1970).

Paulson, G. D., R. G. Zaylskie, M. V. Zehr, C. E. Portnoy, and V. J. Feil: Metabolites of carbaryl (1-naphthyl methylcarbamate) in chicken urine. J. Agr. Food Chem. 18, 110 (1970).

Plimmer, J. R., P. C. Kearney, H. Chisaka, J. B. Yount, and U. I. Klingebiel: 1,3-Bis(3,4-dichlorophenyl) triazine from propanil in soils. J. Agr. Food Chem. 18, 859 (1970).

Pritchard, J. G.: Positive-ion fragmentation mechanisms of some organophosphorus esters. Org. Mass Spectrom. 3, 163 (1970).

Robbins, J. D., J. E. Bakke, and V. J. Feil: Metabolism of benzo [b] thien-4-yl methylcarbamate (Mobam) in dairy goats and a lactating cow. J. Agr. Food Chem. 18, 130 (1970).

Rosen, J. D., D. J. Sutherland, and M. A. Q. Khan: Properties of photoisomers of heptachlor and isodrin. J. Agr. Food Chem. 17, 404 (1969).

Ross, J. A., and B. G. Tweedy: Mass spectra of chloro-, aminochloro-, and ethylaminochloro-s-triazines. Org. Mass Spectrom. 3, 219 (1970).

Saha, J. G., and Y. W. Lee: Isolation and identification of the components of a commercial chlordane formulation. Bull. Environ. Contamination Toxicol. 4, 285 (1969).

Schwemmer, B., W. P. Cochrane, and P. B. Polen: Oxychlordane, animal metabolite of chlordane: Isolation and synthesis. Science 170, 1087 (1970).

Stark, A.: Analysis of pentachlorophenol residues in soil, water, and fish. J. Agr. Food Chem. 17, 871 (1969).

Suggs, J. E., R. E. Hawk, A. Curley, E. L. Boozer, and J. D. McKinney: DDT metabolism: Oxidation of the metabolite 2,2-bis(p-chlorophenyl) ethanol by alcohol dehydrogenase. Science 168, 582 (1970).

Tweedy, B. G., C. Loeppky, and J. A. Ross: Metabolism of 3-(p-bromophenyl)-1-methoxy-1-methylurea (metobromuron) by selected soil microorganisms. J. Agr. Food Chem. 18, 851 (1970).

White, E. R., W. W. Kilgore, and G. Mallett: Phygon. Fate of 2,3-dichloro-1,4-naphthoquinone in crop extracts. J. Agr. Food Chem. 17, 585 (1969).

Zink, P.: Identification of various chlorinated herbicidal phenoxycarboxylic acids by means of mass spectrometry. Arch. Toxikol. 25, 1 (1969); through Chem. Abstr. 71, 79263 (1969).

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Determination of Metabolic and Hydrolytic Products of Organophosphorus Pesticide Chemicals in Human Blood and Urine

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The procedure of St. John and Lisk for the determination of the hydrolytic metabolites of organophosphorus insecticides in cow urine has been modified for application to human blood and urine. The extracting solvent, a 1 to 1 mixture of acetonitrile and diethyl ether, resulted in an average recovery of 90, 89, and 87%, of the six dialkyl phosphates from water, urine, and blood, respectively. The interference from inorganic phosphates was eliminated by making the methyl and ethyl derivatives of the dialkyl phosphates. The use of a column packed with 20% Versamid on Gas Chrom Q allowed for the simultaneous determination of the six major metabolites of organophosphorus and thiophosphorus insecticides. The level of alkyl phosphates excreted in the urine of exposed individuals was considerably higher than in the nonexposed group.

The metabolism and urinary hydrolysis of organophosphorus pesticide chemicals in mammals results in the excretion of a variety of alkyl phosphates. These include the salts of dimethyl or diethyl phosphate, thiophosphate, or dithiophosphate. The separation and quantitation of such products in blood or urine is of value in estimating the extent of exposure to the parent pesticide chemical.

The methylation and ethylation of mono-, and dimethyl phosphates and the mono- and diethyl phosphates produced the esters dimethyl monoethyl phosphate, diethyl monomethyl phosphate, trimethyl phosphate, and triethyl phosphate. The preparation of both the methyl and ethyl esters can be used for the definite identification of the various mono- and dialkyl phosphates by gas chromatography (Stanley, 1966).

Gas chromatographic analysis of the methylated metabolites, utilizing a thermionic detector, has been described (Gutenmann *et al.*, 1968; St. John and Lisk, 1968). An investigation of the applicability of this procedure to human urine and blood samples has resulted in changes in the extraction, alkylation, and chromatographic steps which make the method more sensitive, eliminate interferences from inorganic phosphates, and allow for the simultaneous determination of six major metabolites or hydrolysis products of organophosphorus insecticides.

EXPERIMENTAL

Solvents and Reagents. Extracting solvent, mixture of acetonitrile and diethyl ether (1 to 1).

N-methyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich Chemical Co., Milwaukee, Wis.).

N-ethyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich Chemical Co., Milwaukee, Wis.).

Dimethyl phosphate (DMP) 10 μg./5 ml.
Dimethyl thiophosphate (DMTP) 10 μg./5 ml.
Dimethyl dithiophosphate (DMDTP) 25 μg./5 ml.
Diethyl phosphate (DEP) 5 μg./5 ml.
Diethyl thiophosphate (DETP) 10 μg./5 ml.
Diethyl dithiophosphate (DEDTP) 10 μg./5 ml.
Trimethyl phosphate (TMP), in hexane, 1 μg./ml.
Triethyl phosphate (TEP), in hexane, 1 μg./ml.
Potassium dihydrogen phosphate, in water, 100 μg./ml.

Prepare the solutions of the potassium salts of the dialkyl phosphates in water for use as standard solutions and store them in a freezer. Frozen solutions are stable for about three months.

Diazoalkane reagent (Stanley, 1966), prepared as follows: Dissolve 2.3 grams of potassium hydroxide in 2.3 ml. of distilled water in a 125-ml. Erlenmeyer flask, cool, and add 25 ml. of ethyl ether. Cool the flask in a freezer. Carry out the following preparation in a highly efficient hood. Add either 1.5 grams of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine for diazomethane, or 1.6 grams of *N*-ethyl-*N'*-nitro-*N*-nitroso guanidine for diazoethane in small portions over a period of a few minutes to the flask, and shake the flask vigorously after each addition. Decant the ether layer into a bottle capped with a "poly seal" cap (A. H. Thomas Catalog No. 2849-E) and store in a freezer. (Caution: Diazoalkanes are skin irritants and carcinogens.) Do not use a ground-glass-stoppered bottle. (If it is kept in a tightly capped bottle, the diazoalkane solution may be stored at −20 °C. for a week.) This procedure gives about 16 ml. of ether solution. The diazoalkane can be prepared in a larger quantity by increasing the quantities of the reagents used but the proportions must not be changed because a higher diazoalkane concentration might result in an explosion. Do not use etched or scratched glassware and avoid exposure to strong light.

Apparatus. FLAME PHOTOMETRIC DETECTOR. (Melpar Corp., Falls Church, Va., available from MicroTek Instrument Co., Baton Rouge, La.) equipped with the 394-mμ and 526-mμ filters (to determine sulfur and phosphorus compounds, respectively) attached to a MicroTek MT 220 gas chromatograph.

MODIFICATIONS OF GAS CHROMATOGRAPH. (a) Equip bucking control on electrometer Type E-2 with a 10-megohm Victoreen resistor.

(b) Install Carle switching valve No. 2011 with automatic actuator No. 2050 interfaced between gas chromatographic column and flame photometric detector. (The valve, which is heated with a 432-watt, $2^{1}/_{2}$- × 24-inch insulated heating tape, permits interchange of column effluent and nitrogen purge). Adjust the nitrogen purge flow rate to equal the flow from the gas chromatographic column so that when an interchange of flows is made for the purpose of venting solvent, no change is observed in the recorder base line. This arrangement avoids extinguishing the flame when sample injections are made.

GAS CHROMATOGRAPHIC COLUMN. Pack an aluminum column ($^{1}/_{4}$-inch o.d. × 12 feet) with 60- to 80-mesh Gas

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Department of Health, Education, and Welfare, Public Health Service, Consumer Protection and Environmental Health Service, Food and Drug Administration, Bureau of Science, Division of Pesticides, Perrine Primate Research Branch, P.O. Box 490, Perrine, Fla. 33157

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Chrom Q coated with 20% Versamid 900. (On-column injection is used for all analyses.)

Column Packing. Dissolve Versamid 900 in hot 1 to 1 (v./v.) mixture of chloroform and *n*-butanol. (The volume of the mixed solvent should be just enough to produce a slurry when added to the Gas Chrom Q.) Place the weighed Gas Chrom Q in an evaporating dish, then add the dissolved Versamid and mix gently. Leave the evaporating dish in a hood overnight or until all the solvent has evaporated. Place in an oven overnight at 150 °C. Do not make any attempt to sieve the preparation. (The presence of agglomerates does not affect the efficiency of the column packing.) Condition the prepared column at 200 °C. for four days.

Disposable Capillary Pasteur Pipets were also used.

Methods. Preparation of Standard Curves. Dialkyl Phosphates. Pipet an aliquot of each of the dialkyl phosphate standard solutions into a 100-ml. centrifuge tube. Dilute the combined aliquots of 5 ml. with distilled water. Add 15 grams of sodium chloride and exactly 10 ml. of the extracting solvent (1 to 1 v./v. mixture of acetonitrile and diethyl ether) to the centrifuge tube. Add 1 ml. of 5*N* HCl, stopper the tube, and proceed with immediate, vigorous mixing for 1 minute with the aid of a Vortex-Genie mixer (Scientific Industries, Inc.). Centrifuge for 1 minute to obtain complete separation of the two layers. Immediately pipet an aliquot (1 to 9 ml.) of the organic solvent layer into a 15-ml. glass-stoppered graduated centrifuge tube. Avoid prolonged contact of the sample with the 5*N* HCl solution to prevent the decomposition of the dialkyl phosphate. Concentrate aliquots larger than 2 ml. to 1 to 2 ml. with a gentle stream of nitrogen. Alkylate by adding either 10 drops of methanol and 2 ml. of diazomethane solution or 10 drops of ethanol and 2 ml. of diazoethane solution to the ether concentrate. Allow the mixture to stand for 20 minutes in a well-ventilated hood. Bubble dry nitrogen through the solution using a disposable pipet connected to a nitrogen cylinder. When the yellow color of the diazoalkane disappears, cease bubbling nitrogen and dilute the standard solution to 2 to 10 ml. with either methanol or ethanol, depending on the alkyl derivative being made. Inject 5 to 50 µl. into the gas chromatograph.

Inorganic Phosphates. Pipet an aliquot of KH_2PO_4 standard solution (0.01–0.1 ml.) into a 10-ml. graduated test tube. Add one drop of concentrated HCl and 10 drops of either methanol or ethanol followed by 2 ml. of the corresponding diazoalkane solution. Proceed as described above for dialkyl phosphates.

Trimethyl Phosphate and Triethyl Phosphate. Inject appropriate amounts of the standard solutions directly into the gas chromatograph.

Extraction of Blood and Urine. Analyze each of two 5-ml. aliquots of a blood or urine sample in the following manner: Pre-extract a 5-ml. aliquot of blood or urine contained in a 100-ml. centrifuge tube, with two 5-ml. portions of diethyl ether and discard the extracts. Pre-extraction is necessary only when the urine has been contaminated with feces as would be the case in using this procedure on urine collected from experimental animals housed in cages. The pre-extraction does not remove any of the metabolites described in this paper. Add sodium chloride and extracting solvent, acidify, and proceed as described under preparation of standard curves.

Alternatively, if alkyl phosphate levels permit, extract one 5-ml. portion of sample, and pipet two equal aliquots of the organic solvent extract into two 15-ml. graduated centrifuge tubes. (It is necessary to methylate and ethylate each sample; see Results and Discussion.) After complete removal of the diazoalkane, inject 10 µl. of the solution into the gas chromatograph as a trial sample to determine whether the sample must be diluted or concentrated before proceeding to the gas chromatographic step. As in the case of the standards, dilute the methylated derivative with methanol and the ethylated derivative with ethanol. Use injections of 5 to 50 µl. from a final volume of 2 to 50 ml. for satisfactory results.

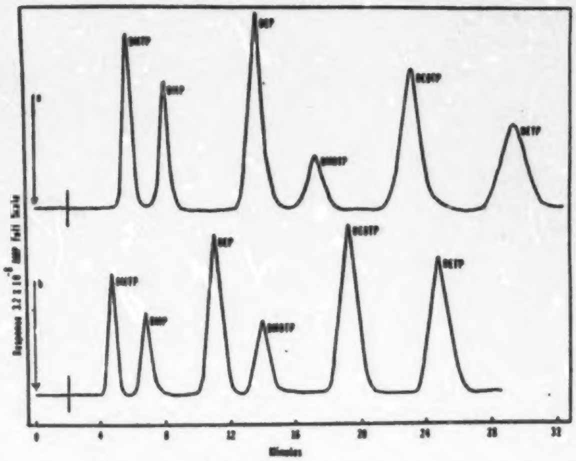

Figure 1. Chromatograms of trialkyl phosphate esters using the phosphorus filter, 526 mµ

a. Ethylated: 6.5 ng. DMTO; 8 ng. DMP; 4 ng. DEP; 45 ng. DMDTP; 10.5 ng. DEDTP; 8.5 ng. DETP.
b. Methylated: 11 ng. DMTP; 5.7 ng. DMP; 3.2 ng. DEP; 35 ng. DMDTP; 10 ng. DEDTP; 8.4 ng. DETP

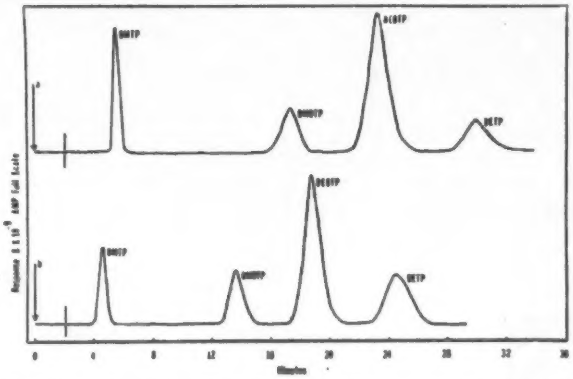

Figure 2. Chromatograms of trialkyl phosphate esters using the sulfur filter, 394 mµ

a. Ethylated: 25 ng. DMTP; 120 ng. DMDTP; 20 ng. DEDTP; 20 ng. DETP
b. Methylated: 50 ng. DMTP; 120 ng. DMDTP; 20 ng. DEDTP; 20 ng. DETP

Gas Chromatography. Inject appropriate aliquots of the sample and standard extracts into the gas chromatograph. Set the operating temperatures of the column, injection block, detector, transfer lines, and switching valve to 142, 143, 130, 145, and 145 °C., respectively. Adjust the gas flow rates for nitrogen (carrier), nitrogen (purge), hydrogen, air, and oxygen to 90, 90, 180, 80, and 10 ml. per minute, respectively.

RESULTS AND DISCUSSION

Chromatograms in Figures 1 and 2, of the ethylated and methylated mixtures of dialkyl phosphates, were obtained with the use of the phosphorus filter and sulfur filter, respectively.

Table I. Isothermal Gas Chromatography of Trialkyl Phosphates with Phosphorus Flame Photometric Detection System

Trialkyl Phosphate	Relative Retention Time	Detector Sensitivity (4/1 Signal-to-Noise Ratio), Nanograms	Limit of Detectability, P.P.M.
TMTP	0.70		
TMP	1.00		
DEMMP	1.72	0.4	0.01
TMDTP	2.09	15.0	0.20
DEMMDTP	2.98	1.0	0.02
DEMMTP	4.12	1.0	0.02
DMMETP	0.71	1.5	0.02
DMMEP	1.00	1.5	0.02
TEP	1.75		
DMMEDTP	2.18		
TEDTP	3.08		
TETP	3.96		

TMTP = Trimethyl thiophosphate; TMP = Trimethyl phosphate; DEMMP = Diethyl monomethyl phosphate; TMTP = Trimethyl dithiophosphate; DEMMDTP = Diethyl monomethyl dithiophosphate; DEMMTP = Diethyl monomethyl thiophosphate; DMMETP = Dimethyl monoethyl thiophosphate; DMMEP = Dimethyl monoethyl phosphate; TEP = Triethyl phosphate; DMMEDTP = Dimethyl monoethyl dithiophosphate; TEDTP = Triethyl dithiophosphate; TETP = Triethyl thiophosphate.

Table II. Recovery of Dialkyl Phosphates from Water, Urine, and Blood

Dialkyl Phosphate	Added, µg.	Recovered from 5 ml, µg[a]		
		Water	Urine	Blood serum
DMTP	10	9.9	9.9	9.0
DMP	5	4.2	4.2	4.3
DEP	5	4.7	4.7	4.6
DMDTP	50	42.0	38.0	37.0
DEDTP	10	8.3	8.3	8.2
DETP	10	10.0	10.0	9.5

[a] Average of three determination.

The following dialkyl phosphate derivatives were found to give a linear response in the range of 0 to 10 ng.: Ethylated DMTP and DMP; methylated DEP, DEDTP, DETP. On the other hand, the methylated DMDTP was found to give a linear response in the range of 0 to 100 ng. Quantitation was made using the phosphorus filter.

A comparison of the standard curves reveals that the ethylated DMP and DMTP produce a greater detector response than the corresponding methylated compounds. Conversely, the methylated derivatives of DEP, DMDTP, and DETP produce a greater detector response than the corresponding ethylated derivatives. For this reason, quantitation is based on the methylated DEP, DMDTP, DEDTP, DETP, and the ethylated DMTP and DMP. However, it should be pointed out that this difference in sensitivity is not the only reason for methylating and ethylating each sample. The extraction step removes inorganic phosphates from the sample. The phosphate, in turn, converts to either TMP or TEP, depending on the diazoalkane used for derivatization. In one case, the TMP interferes with methylated DMP; in the other, the TEP interferes with the ethylated DEP. This problem can be circumvented by splitting the extract and making both derivatives. The TMP produced during methylation is vented in much the same manner as the solvent peak; likewise, in the case of ethylation, the TEP peak is vented.

Table I lists the relative retention times, detector sensitivity, and limits of detectability for the methylated and ethylated dialkyl phosphates using the phosphorus filter.

In order to determine the efficiency of the alkylation step, a quantitative comparison was made between commercially available TMP and TEP and the corresponding derivatives produced by methylating DMP or ethylating DEP. In both cases, the standard curves were superimposable, indicating a 100% conversion. Furthermore, the methylation or ethylation of acidified KH_2PO_4 produced TMP and TEP which were quantitatively indistinguishable from the standard curves discussed above.

Primary standards for the other trialkyl phosphates discussed were not available and, therefore, they were not tested. However, it was assumed that the efficiency of derivatization is the same for these compounds as for those actually used.

As shown in Table II, the average recovery of six dialkyl phosphates from water, urine, and blood was 90, 89, and 87%, respectively.

The ability to interchange the sulfur and phosphorus filters in the single detector or the use of the base assembly for dual phototube operation with both filters (Bowman and Beroza, 1968) greatly enhances the specificity of this method. Suspected thiophosphate can be confirmed with the sulfur filter by simply increasing the concentration of the compound injected into the gas chromatograph by a factor of 5 to 10. With the sulfur filter in the detector, up to 200 ng. of TMP or TEP can be injected without producing a significant detector response.

The monoalkyl phosphates undergo derivatization by this technique to produce dimethyl monoalkyl phosphates on methylation or diethyl monoalkyl phosphates on ethylation—*i.e.*, methylated DEP is used as a standard for ethylated monomethyl phosphate metabolite. A mixture of monomethyl and dimethyl phosphate after ethylation would yield DEMMP and DMMEP compounds which are readily separated on the Versamid gas chromatographic column.

The results for any particular compound can be confirmed by forming the *n*-propyl derivative as in the method described for methylation and ethylation, except that *N*-*n*-propyl-*N'*-nitro-*N*-nitrosoguanidine is used as the diazoalkane precursor. The *n*-propyl derivative can also be used to circumvent the interference by inorganic phosphates except that the analysis time would be unduly lengthened.

Urine and blood serum samples from different individuals were analyzed for their alkyl phosphate content with the results grouped into three categories, shown in Table III. The classification is somewhat arbitrary and based on information supplied by Health Officers. The first group of subjects was exposed to dichlorvos, malathion, and carbophenothion, and the samples were taken 14 days after the last exposure. Members of the second group were operators of a nursery and landscaping organization and the third were nonexposed individuals. The results of the analysis of these samples are presented as a first indication of the potential of this method for determining the extent of exposure to organophosphorus compounds. Additional experiments with humans and animals are in progress in order to establish this relationship more precisely.

Figures 3 and 4 illustrate chromatograms of methylated and ethylated urine extract of an exposed individual.

Equipping the gas chromatograph with an automated switching valve greatly simplifies the operation of the flame photometric detector. The flame and, hence, the base line are not disturbed during the analysis because excess solvents

Table III. Urine and Blood Serum Alkyl Phosphate Content of Exposed and Nonexposed Individuals Concentration, P.P.M.

Degree of Exposure[a]	MMP[b]	DMTP	DMP	DEP	DMDTP	DEDTP	DETP	Serum[c] DEP	Serum[c] DMDTP
(1) Heavy	*N.D.*[d]	*N.D.*	0.13	0.20	1.52	*N.D.*	0.21	0.20	3.50
	0.02	0.11	0.12	1.72	1.20	*N.D.*	0.18	0.15	3.40
	N.D.	*N.D.*	*N.D.*	0.12	1.86	*N.D.*	*N.D.*	0.20	3.00
(2) Moderate	*N.D.*	*N.D.*	0.05	0.03	0.69	*N.D.*	0.04	*N.D.*	0.11
	0.03	*N.D.*	*N.D.*	*N.D.*	0.73	*N.D.*	0.04	*N.D.*	...
	N.D.	*N.D.*	*N.D.*	0.02	1.76	*N.D.*	*N.D.*	*N.D.*	0.15
	N.D.	*N.D.*	*N.D.*	*N.D.*	0.56	*N.D.*	*N.D.*	...	...
	N.D.	*N.D.*	*N.D.*	0.05	0.96	*N.D.*	0.04	...	0.16
	0.02	*N.D.*	*N.D.*	0.07	1.40	*N.D.*	*N.D.*	*N.D.*	0.12
	0.03	0.04	*N.D.*	0.04	1.47	*N.D.*	0.04	*N.D.*	0.16
	0.03	*N.D.*	*N.D.*	*N.D.*	1.22	*N.D.*	0.04	...	...
(3) Light or None	*N.D.*	*N.D.*	*N.D.*	0.02	0.40	*N.D.*	*N.D.*	*N.D.*	*N.D.*
	N.D.	*N.D.*	*N.D.*	0.04	0.38	*N.D.*	*N.D.*	*N.D.*	*N.D.*
	N.D.	*N.D.*	*N.D.*	*N.D.*	0.22	*N.D.*	*N.D.*	*N.D.*	*N.D.*
	N.D.	*N.D.*	*N.D.*	*N.D.*	<0.20	*N.D.*	*N.D.*	*N.D.*	*N.D.*

[a] Selection category is arbitrary and based on information supplied by Health Officers.
[b] Ethylated monomethyl phosphate, calculated using a methylated diethyl phosphate standard.
[c] Other alkyl phosphates were not found in the serum samples.
[d] Not determined.

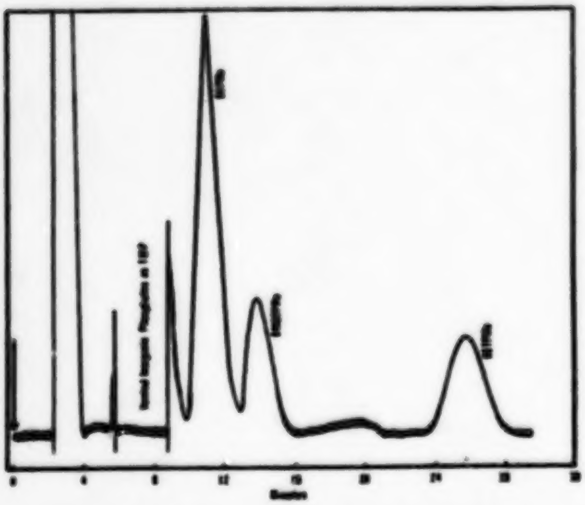

Figure 3. Chromatogram of methylated urine extract

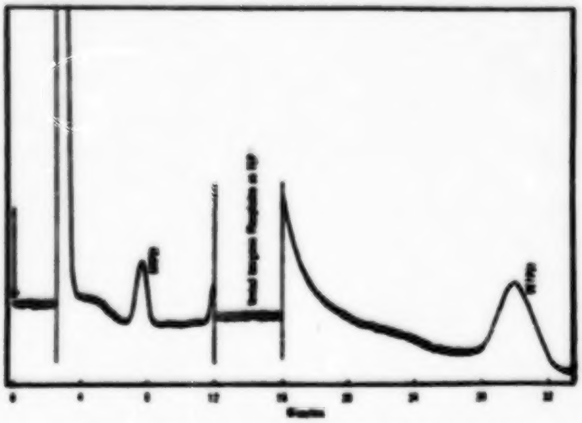

Figure 4. Chromatogram of ethylated urine extract

and other compounds are vented to the atmosphere with essentially no interruption of any of the gases flowing to the detector.

Aside from the specific application cited in this paper, the method described probably can be used in the following applications:

Determine the rate of decomposition of organophosphorus pesticide formulations through analysis of the resulting alkyl phosphates.

In both animal and plant tissue extracts, determine the presence of organothiophosphates and their corresponding oxons through alkaline hydrolysis and subsequent derivatization.

Determine the presence of gas chromatographically unstable organophosphorus insecticides (vinyl phosphates, TEPP, etc.) in a variety of substrates *via* a preliminary alkaline hydrolysis, followed by derivatization and quantitation using a phosphorus detector.

Study metabolism reactions such as dealkylation, isomerization, and oxidation of organophosphorus pesticides without recourse to radioactive labelled compounds.

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LITERATURE CITED

Bowman, M. C., Beroza, M., *Anal. Chem.* **40,** 1448 (1968).
Gutenmann, W. H., St. John, J. E., Jr., Lisk, D. J., J. Agr. Food Chem. 16, 45 (1968).
St. John, L. E., Jr., Lisk, D. J., J. Agr. Food Chem. 16, 48 and 408 (1968).
Stanley, C. W., J. Agr. Food Chem. 14, 321 (1966).

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